This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

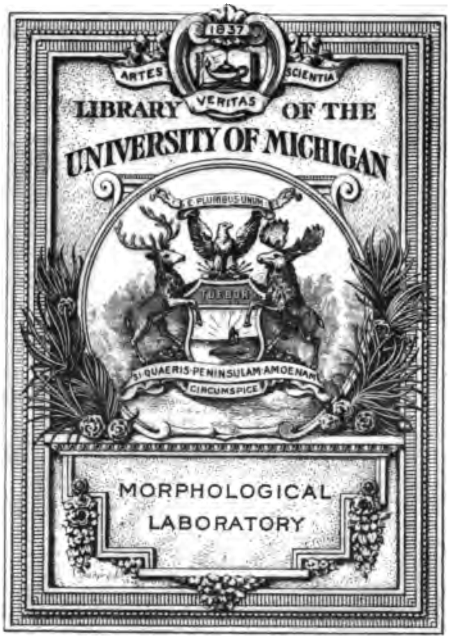
About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

QH
499
.Z49

BUHR B

a39015 00007868 6b



LIBRARY OF THE
UNIVERSITY OF MICHIGAN

MORPHOLOGICAL
LABORATORY

THE ~~GIFT~~ OF
Exchange

SCIENCE LIBRARY
Q.A
499
.Z49

2-725

THE UNIVERSITY OF CHICAGO
Founded by John D. Rockefeller

Compensatory Regulation

A Dissertation Submitted to the Faculty of the Ogden Graduate School of Science
in Candidacy for the Degree of Doctor of Philosophy, Department of Zoölogy

By
CHARLES ZELENY

May 17, 1904

Reprinted from the Journal of Experimental Zoölogy
Volume II, No. I
Baltimore, April, 1905

COMPENSATORY REGULATION.

BY

CHARLES ZELENY.

General Introduction 2
Data 5
- I. The Leaflets of the Compound Leaf 5
- II. The Rate of Regeneration of the Arms in the Brittle-star, Ophioglypha lacertosa 7
  - 1. Introduction 7
  - 2. Method 8
  - 3. Data 14
  - 4. Discussion 16
- III. The Opercula of Serpulids 18
  - 1. Comparative Anatomy 19
    - 1. The genus Hydroides 19
    - 2. Other genera of Serpulids 24
    - 3. Distribution of the Opercula between Right and Left Sides 32
    - 4. Exceptional Degrees of Development and One Case of a Supernumerary Operculum 37
  - 2. Development of Opercula—
    - 1. Ontogenetic Development 38
      - *a.* Introduction 38
      - *b.* Historical Review 39
      - *c.* Observations 41
      - *d.* Summary of Data and Discussion 51
    - 2. Regeneratory Development 54
      - *a.* Introduction 54
      - *b.* Unoperated Condition of the Opercula in H. dianthus 55
      - *c.* Operations on Functional Operculum 55
      - *d.* Operations on Rudimentary Operculum 58
      - *e.* Operations on Both Opercula 59
      - *f.* Body Cut in Two 62
      - *g.* Progressive Changes in Opercula 65
      - *h.* Experiments on Group V 65
      - *i.* Experiments on Group IV 67
      - *j.* Experiments on Group VI 69
      - *k.* Discussion of the Data 71
    - 3. Probable Phylogenetic Development 72
  - 3. Comparison of Regeneratory, Ontogenetic and Probable Phylogenetic Development 74
  - 4. General Discussion of the Facts of Compensatory Regulation in the Opercula of Serpulids 76

142750

IV. The Rate of Differentiation during Regeneration of the Opercula in the Serpulid, Apomatus 77
V Regulation of the Rate of Growth and Nature of Differentiation during Regeneration of the Chelæ of Gelasimus and Alpheus 81
1. Introduction 81
2. Gelasimus pugilator 81
3. Alpheus dentipes 85
General Discussion 96

GENERAL INTRODUCTION.

That the development of an organ is dependent both quantitatively and qualitatively not only upon the factors inherent in its basis but also upon influences exerted by other parts of the body, is a fact that has been especially emphasized by much of the recent work in experimental embryology. The relative proportion of the two influences varies in individual cases and its determination has served as the objective point in various experimental studies. The most valuable method in attacking these problems has undoubtedly been the one in which the regulation following mutilation of the organism or change in its environment has been observed. Assuming an interaction between the parts of an organism, which is a necessary accompaniment of the idea of their correlation, we must consider the animal or plant as a system in equilibrium very nearly stable at certain periods, as in adults, and with a unilateral instability at other periods, as in embryonic development. This statement must apply not only to the organism as a whole but to all its parts and groups of parts individually.

A disturbance of the normal relations of the parts, such as is brought about by the removal of one of them or by the changing of the external environment, must lead to changes which if the system is not too rigid can give important light upon the normal relations of the parts. This is the fundamental consideration which lies at the base of all experimental biology and in the broad sense of the term all such studies are studies in compensatory regulation.

Of special importance in the present consideration is the fact that while the parts of an organism and their interactions are continually undergoing change, especially during the period of unilateral instability, the so-called embryonic period, these changes are of a remarkably constant character for individuals of a species, notwithstanding environmental changes within a fairly wide limit. At the same time members of different species are constantly dissimilar notwithstanding similar external environments in many cases.

Disturbances of the system of an organism as above conceived may be conveniently grouped under two heads: First, disturbances in external environment, not considered here, and second, direct internal disturbances. As direct internal disturbances may be classed all mutilations. The removal of a part causes disturbances in the remaining parts which as the result of a new mode of interaction adjust themselves to a new stable system. As an example may be mentioned the rearrangement of the leaflets of a compound palmate leaf after removal of one of them. When complete regeneration takes place the new system may finally regain its original condition. However, during the intermediate stages while the regeneration of the organ is in progress a new condition of interaction must be present and this may lead to changes both in the old parts and in the new regenerating ones, so that the new stable equilibrium is very different from the old.

In a paper on the dimensional relations of the members of compound leaves, the writer has described the changes taking place in the members of a compound leaf after removal of one of the leaflets at an early stage. In this case there was no regeneration and the effects of the operation were confined to position and length changes in the remaining members. The results of these experiments will be considered briefly in a separate section at the beginning of the following discussion.

The present paper will deal, except for the above mentioned experiments, with cases among animals where, as a result of the operation, the character of regeneration of an organ varies with the character of the remainder of the system as in the arms of Ophioglypha and the opercula of Apomatus, or where there is an evident change not only in the regenerating part but also in other parts of the system, so that the final state of equilibrium differs from the original condition, as in the opercula of Serpulids and the chelæ of Alpheus. The experiments on Ophioglypha have already been described in a preliminary paper (Zeleny, '03a), and likewise some of the experiments on the Serpulid, Hydroides dianthus (Zeleny, '02).

It is the plan of the present paper to take up the different groups of experiments on compensatory regulation in turn and to connect them by a final general discussion. The individual groups of experiments are thus quite independent in the descriptive portion.

The work was carried on at the Woods Hole Biological Laboratory in the summer of 1901, at the Cold Spring Harbor Laboratory in 1902, and at the Naples Zoölogical Station from September, 1902, to June, 1903. I wish to express my great obligation to Prof. C. B. Davenport, to Prof. E. B. Wilson and to Prof. T. H. Morgan, for inspiration and aid in carrying out the work, and to the members of the staffs at the three laboratories where it was done, for their uniform kindness in giving every convenience in the course of the investigation.

The data will be considered in five sections as follows:

I. In the *first* section (p. 5) the experiments on the leaflets of the compound leaf will be briefly referred to as constituting a case of regulation of a system in which there is no regeneration of the removed part. The readjustment is here confined to the uninjured portions and the assumption of an interaction between the members of the leaf constitutes an important factor in the explanation of the changes that take place.

II. In the *second* section (p. 7) the rate of regeneration of the arms of the brittle-star, Ophioglypha, is taken up and studied with special reference to the influence which parts of the animal away from the injured surface have upon the nature of the regeneration at that surface.

III. The *third* section (p. 18) consists of experiments on the opercula of the Serpulids made with a view to the analysis of the factors involved in the control of the asymmetry of these animals. Observations on the comparative anatomy of the opercula and on their ontogenetic development made with special reference to the problem of compensatory regulation are included.

IV. The *fourth* section (p. 77) contains observations on the regulation of the rate of differentiation in the regeneration of the opercula of the Serpulid, Apomatus ampullifera. The influence of the removal of the posterior part of the body upon the opercula is taken up. The experiments are put into a section separate from the main one on the opercula of Serpulids because they are not directly concerned with the interaction of the two lateral sides of the body, *i. e.*, with the factors controlling the asymmetry of the opercula, but rather with the influence of the presence or absence of the posterior regions of the body upon the process as a whole.

V. The *fifth* section (p. 81) gives an account of experiments on the regeneration of the chelæ of the Decapod Crustaceans, Gelasimus and Alpheus, with reference, *first*, to the problem of control of the asymmetry of the chelæ in the male Gelasimus and in the male and female Alpheus; *second*, with reference to the general problem of the influence of parts away from an injured surface upon the character of the regeneration at that surface; and, *third*, with reference to the influence of the character of the operation upon the moult period.

Finally, all the data are brought together in a general discussion of the facts of compensatory regulation and their relation to the point of view which considers the organism as a system of mutually interacting parts (p. 96).

DATA.

I. THE LEAFLETS OF THE COMPOUND LEAF.

The simplest instance of the application of the method employed in the present paper is furnished by the experiments on the compound leaf of the palmate type, as described in my paper on "The Dimensional Relations of the Members of Compound Leaves." It will not be necessary to go into the details of that paper but a sample result may be of value, because the case there described is a pure instance of change in the uninjured organs without regeneration of the injured one. The main point of the experiments may be briefly illustrated by the following quotation from the introduction as given there:

> The individual members of the compound leaf as well as of other parts of the plant respond to stimuli in a definite way. Each member is, however, limited in its reaction by its mechanical and organic relations to the other parts of the leaf. This limitation is mutual and as a result of it we get an equilibrium of forces which results in a configuration more or less definite for each species. As a further consequence each member must respond not as a unit but as part of a system. If now we have a system of this kind with a definite configuration due to the mutual interaction of its members and we remove one of the component parts, we must get a disturbance of the equilibrium leading to changes in the relations of the remaining

parts limited only by the extent to which the rigidity of the skeletal structures may counteract such a tendency. We may in this manner get at the forces which are active in correlation at the time of and subsequent to the operation. The main difficulty with the method must consist in the reaction to the stimulus of the injury itself, a factor which does not enter into the normal relations of the parts.

A sample result will illustrate the method in a more concrete manner. When an asymmetrically placed leaflet of a five-leaved form (white lupine or Virginia Creeper) is removed at the earliest possible stage, the remaining four leaflets take up positions which tend to approach those of the units of a symmetrical *four*-leaved system, the chief change of position being confined to the two leaflets which were asymmetrically placed after the operation. In the case of the Virginia Creeper the resultant change of position was +5°.9 for leaflet A, +12°.2 for leaflet B, +24°.4 for leaflet C, and −3°.2 for leaflet E. (See Fig. 1.) Likewise in a three-leaved system (the red clover) after removal of an asym-

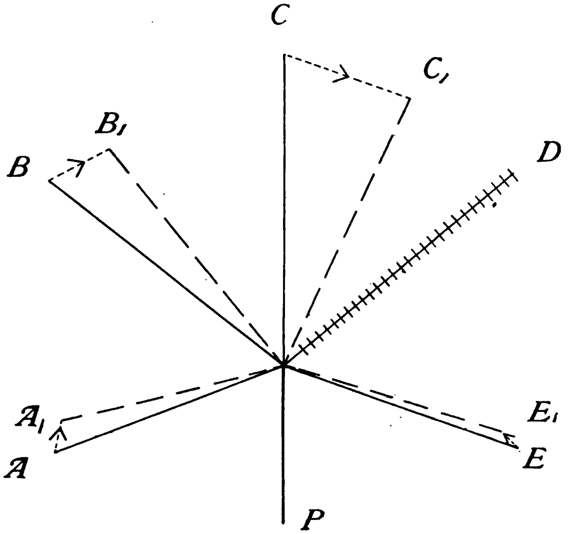


Fig. 1.

Virginia Creeper, Parthenocissus quinquefolia. Diagram of changes in position and length of leaflets of compound leaf after removal of one of their number (× ½). Unbroken lines—Original positions. Dotted lines—Resultant positions. Barred lines—Removed leaflet. *P*—Petiole. Arrow—Direction of movement. *A, B, C, D, E*—Original positions. A_1, B_1, C_1, E_1—Resultant positions.

metrically placed leaflet the resulting two-leaved system tends to be symmetrical with respect to the petiole. This shows very definitely that the normal symmetrical palmate leaf has a definite configuration as a result of the interaction of its units and that the manner of this interaction may be revealed by the removal of a unit, an operation which brings about a *new* mode of interaction leading to a *new* resultant state of equilibrium.

Likewise after such an operation, which is performed at an early stage before the leaflets have fully unfolded, the remaining leaflets do not attain their full normal size, the average decrease for the leaflets of the three species being 6.8 per cent of the normal length. These changes in position and length for the Virginia Creeper are shown in the accompanying figure. (Fig. 1.)

In the following sections the experiments have to deal with more complicated cases because there is a regeneration of a new organ or organs in place of the old, and the changes in the system are the result not only of the interactions of the uninjured parts but also of these upon the regenerating part and in turn of the latter upon the former.

II. THE RATE OF REGENERATION OF THE ARMS IN THE BRITTLE-STAR, OPHIOGLYPHA LACERTOSA.[1]

1. *Introduction.*

In this section we have a case of evident influence of organs situated away from a regenerating surface upon the character of the regeneration at that surface. The influences exerted by the regenerating organ or organs upon the character of the uninjured organs, or the changes produced among the uninjured organs themselves by the new interactions resulting from the new conditions, were not studied because the material was evidently unsuitable for that purpose. The experiments to be described are, therefore, concerned entirely with the rate of regeneration of the arms as influenced by the number of arms removed. Incidentally the variation of the rate of regeneration of the arms with the size (*i. e.*, age [?]) of the animal must be considered.

---

[1] The principal results of this section were given in a preliminary paper already mentioned. (Zeleny, '03b.)

The experiments were performed at the Naples Zoölogical Station during the winter of 1902–03. The common five-armed brittle-star, Ophioglypha lacertosa, was used and five series of experiments, with one, two, three, four and five arms removed, respectively, were kept for 46 days after the operation and no food was supplied to them during the whole period.

The resulting data show that *the rate of regeneration of the arms varies on the one hand with the size of the animal and on the other with the number of removed arms.* Medium-sized individuals show the maximum rate of regeneration and there is a pronounced decrease both for smaller and for larger ones. The second correlation and the one that concerns us especially in the present paper gives *an increase in the rate of regeneration of an arm as we pass from the cases with a smaller to those with a greater number of removed arms.* The series with all five arms missing is excepted in the statement because the animals in this lot in every instance died or showed evidences of decay before the completion of the experiment.

2. *Method.*

Forty-five perfect specimens were divided into five equal groups of nine each, care being taken to distribute them in such a way as to make the sets approximately equivalent as regards size of individuals. The operations consisted in the removal of one or more arms by a transverse cut at the disk level. In the first series one arm was removed, in the second two contiguous arms, in the third three contiguous arms, in the fourth four, and in the fifth five arms. The animals were kept in ten "battery" jars, two for each series and *were not fed during the whole period of the experiment.* Measurements of the lengths of the regenerating arms were taken 22, 33 and 46 days after the operation. As stated above, the specimens of the series where all five arms were removed did not retain their vitality for a sufficient length of time to allow of complete comparison with the others, and they will therefore not be included in the main comparison, though the data concerning them are given in Table I.

The results are given in the accompanying table (Table I), which shows for each of the five series the disk diameters of the specimens and the lengths of the regenerating arm or arms 22, 33 and 46 days after the operation. Where more than one arm

TABLE I.

SERIES I—*One Arm Cut Off.*

Specimen No.	Disk Diam.	22 days	33 days	46 days	Remarks
1	4.8	.15	.2	.0	
2	6.5	.45	.1	.0	
3	6.6	1.4	2.4	3.0	
4	11.0	.6	2.0	2.0	
5	11.2	2.0	3.0	4.0	
6	13.2	.2	2.0	3.1	
7	13.5	—	—	—	dead (22)
8	14.5	1.0	—	—	dead (33)
9	19.8	.0	.0	.0	

SERIES II—*Two Arms Cut Off.*

Specimen No.	Disk Diam.	22 days	33 days	46 days	Remarks
1	6.0	—	—	—	dead (22)
2	6.5	.55	.45	.0	
3	8.7	1.9	1.9	2.0	
4	10.8	1.8	1.6	.65	
5	13.0	—	—	—	dead (22)
6	13.5	1.25	2.3	3.5	
7	14.0	1.8	3.0	3.3	
8	15.0	.85	2.6	4.05	
9	19.3	.0	.0	.0	

SERIES III—*Three Arms Cut Off.*

Specimen No.	Disk Diam.	22 days	33 days	46 days	Remarks
1	5.5	—	—	—	dead (22)
2	6.5	.6	1.05	—	dead (46)
3	9.0	—	—	—	dead (22)
4	11.0	1.15	1.75	3.05	
5	12.5	1.35	2.35	3.2	
6	12.5	2.25	4.25	6.65	
7	13.8	1.1	3.4	4.9	
8	14.3	1.1	1.7	2.15	
9	20.0	.05	.35	1.0	

SERIES IV—*Four Arms Cut Off.*

Specimen No.	Disk Diam.	22 days	33 days	46 days	Remarks
1	4.9	.65	1.0	.45	
2	6.0	1.0	1.65	1.9	
3	8.2	2.45	4.6	6.2	
4	11.3	1.85	3.65	5.3	
5	12.3	2.15	4.3	7.1	
6	12.8	.6	2.1	4.05	
7	12.8	1.55	2.9	4.85	
8	15.2	1.75	3.3	5.55	
9	18.3	1.0	1.75	3.65	

SERIES V—*Five Arms Cut Off.*

Specimen No.	Disk Diam.	22 days	33 days	46 days	Remarks
1	6.0	.6	.1	.0	dying (46)
2	6.5	.76	.1	.0	dying (46)
3	7.0	.6	.46	.0	dying (46)
4	11.0	2.26	2.86	2.66	
5 to 9	—	—	—	—	dead (22)

EXPLANATION OF TABLE I.

The data for the experiments on the regeneration of the arms in Ophioglypha lacertosa after their removal by a cut level with the circumference of the disk. The measurements are in millimeters. In each of the sub-tables the second column gives the disk diameters, the third column the average lengths of the regenerating arms 22 days after the operation, the fourth column the lengths after 33 days and the fifth column after 46 days. In the four last tables (*i. e.*, in all except Series I) the lengths as given are the averages of all the regenerating arms of the individuals. The numbers under "Remarks" represent the time of death (in days) of specimens which did not outlive the experiment.

was removed, *i. e.*, in all except the first series, this length represents the average length of all the regenerating arms of the individual. In some specimens there is a decrease in the length of the regenerated arm from one period to another. This is due to a gradual disintegration and breaking off of the regenerating arm,

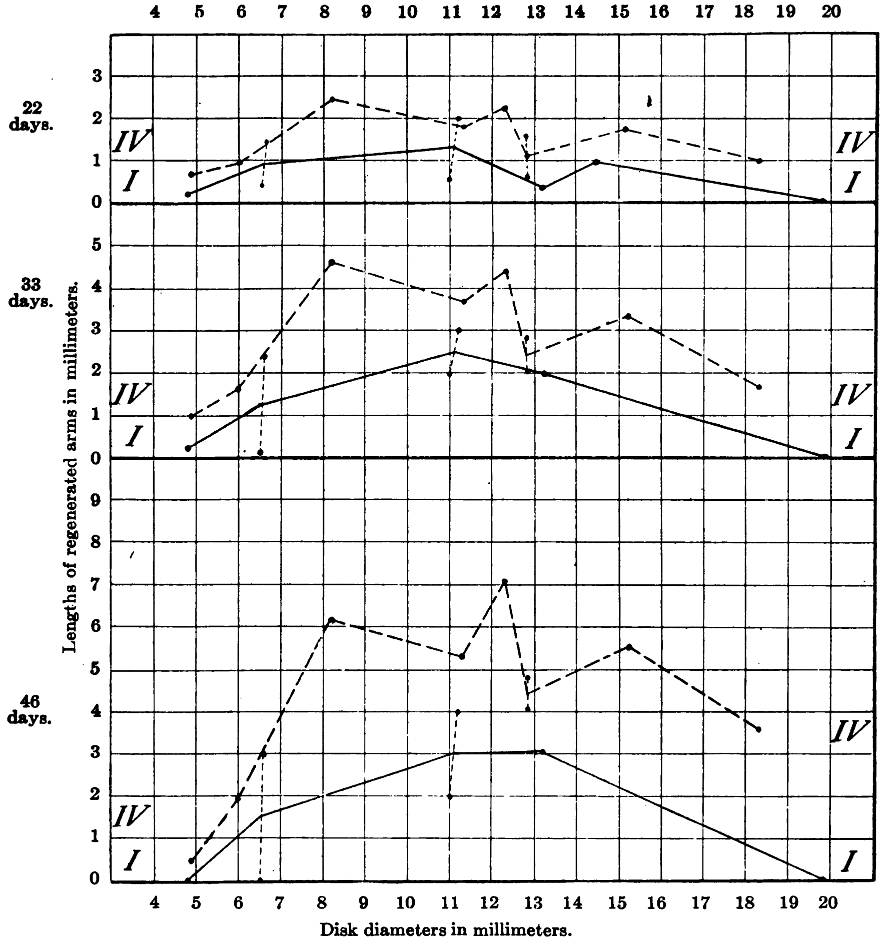


FIG. 2.

Brittle-star, Ophioglypha lacertosa. Comparison of lengths of regenerated arms in Series IV (four arms removed), *dotted lines*, with Series I (one arm removed), *unbroken lines*. Upper curves, 22 days; middle curves, 33 days; and lower curves, 46 days after operation. The lengths of the regenerated arms are distinctly greater in Series IV than in Series I.

a process which begins at the distal end and is usually accompanied by a loss of vitality with the approach of general bodily decay. In Series V, where all five arms were removed, none of the specimens retained its full vitality for the whole 46 days, though one did so for 33 days.

A glance at the table (Table I) is sufficient to show both of the main points brought out in the general statement of the results. In the first place the rate of regeneration of the arms is greatest in medium-sized individuals, decreasing for both the smaller and the larger ones and second, the rate of regeneration is dependent on the number of arms removed. Excepting Series V, where all the arms are removed, there is an evident increase in the rate of regeneration of the arms as we pass from the cases with the fewer to the cases with the greater number of removed arms.

As both these factors enter into the individual measurements the proper relations can best be represented graphically by means of "curves." Such "curves" are shown in Figs. 2 and 3 and the relative rates of regeneration of the arms are also represented for two typical individuals in Fig. 4, which gives two specimens, one from Series I (one arm removed) and the other from Series IV (four arms removed). In each of the individuals in Fig. 4 the condition of the arms 46 days after the operation is represented. Each of the four removed arms of the individual from Series IV has evidently regenerated more rapidly than the single removed arm of the individual from Series I.

In Fig. 2, Series I and IV, with respectively one and four arms removed, are compared. The individual cases are shown by dots. The abscissæ give the sizes of the animals as represented by the disk diameters in millimeters. The ordinates give the lengths of the regenerating arms also in millimeters. In the series where more than one arm was operated on the regeneration length as given is an average of all the regenerating arms of the individual. The individual cases of each series are connected by lines so as to give a basis for comparison of the two groups. The unbroken lines represent the lengths of the members of Series I and the dotted lines those of the members of Series IV. The upper two curves give the measurements as taken 22 days after the operation, the middle pair those at 33 days and the lower pair those at 46 days. In each group the curve showing the rate of regeneration of the arms in the series with four arms removed is well above the one with only one arm removed. Fig. 2 likewise shows the increase in the rate of regeneration of the arms as we pass from the individuals with a smaller disk diameter to those with a medium disk diameter (about 12 mm.) which show the maximum rate. Then there is a decrease in rate of regeneration until indi-

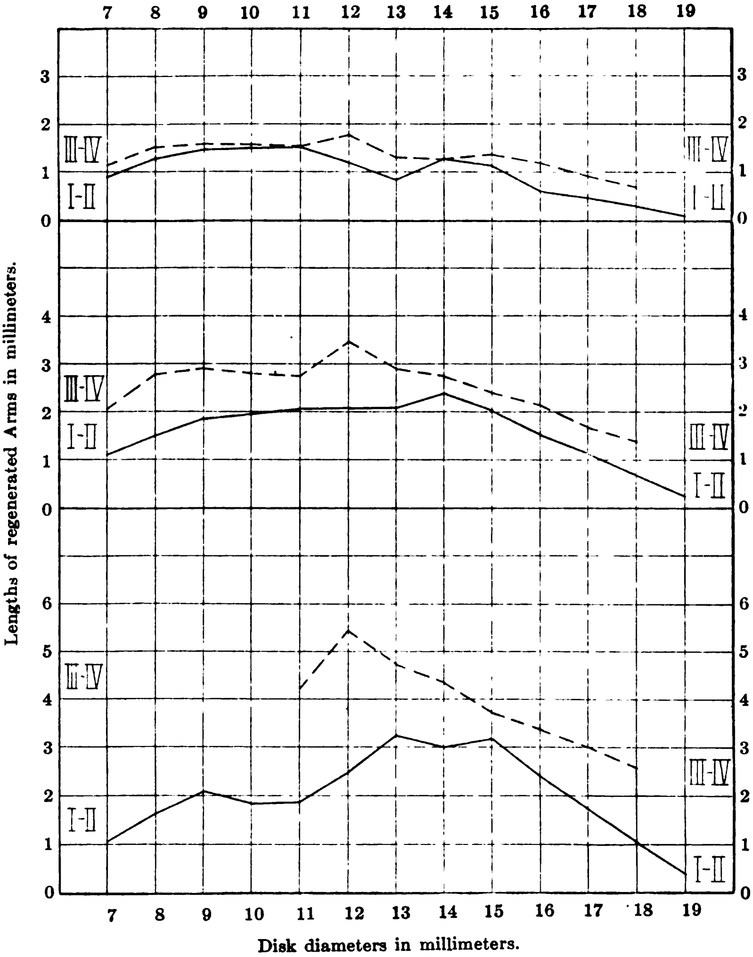


Fig. 3.

Brittle-star, Ophioglypha lacertosa. Comparison of lengths of regenerated arms in Series III and IV combined (three and four arms removed), *dotted lines*, with Series I and II combined (one and two arms removed), *unbroken lines*. Upper curves, 22 days; middle curves, 33 days; lower curves, 46 days after operation. The regenerated arm lengths are greater in Series III and IV than in Series I and II.

viduals of 19 or 20 mm. are reached when the regeneration length for 46 days arrives at a second minimum. A similar relation between rate of regeneration and disk diameter is shown in Fig. 3.

In Fig. 3 all the data for the Series I, II, III and IV are included. It was not, however, possible to put in the individual measurements without confusing the general effect of the curves. Therefore Series I and II are combined in one curve (the unbroken line) and Series III and IV in another (the dotted line). As the individual disk diameters are not exactly equivalent in the different series, it was found convenient in taking the averages for the combination curves to use arbitrarily disk diameters equal to whole millimeters as the points for comparison. The values for such disk diameters are obtained from the separate curves constructed from the individual measurements for each series according to the method shown in Fig. 2. The average curve for Series I and IV in Fig. 2 would be one equidistant between them. The combination curves for Series I and II and Series III and IV, as given in Fig. 3, are constructed on this basis. The unbroken line gives

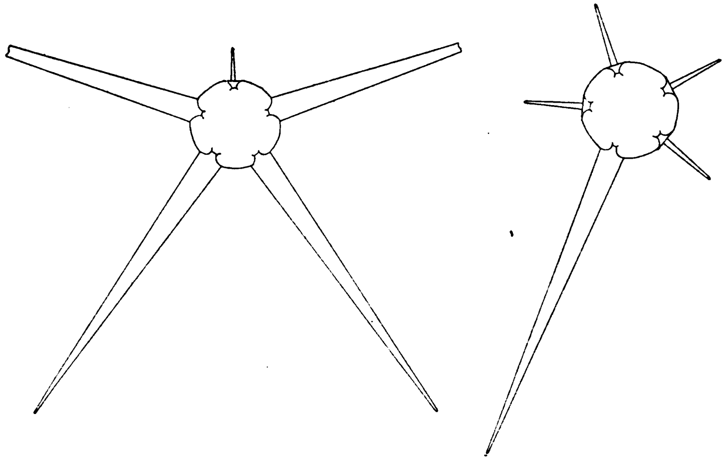


FIG. 4.

Brittle-star, Ophioglypha lacertosa. Left figure—Typical specimen with one regenerating arm. Right figure—Typical specimen with four regenerating arms. Both 46 days after operation. In left specimen two unoperated arms are shortened for lack of space in figure (× $1\frac{1}{5}$).

the average of the former group (one and two arms removed) and the broken line the average of the latter group (three and four arms removed).

3. *Data.*

The curves show very distinctly the correlation between the rate of regeneration on the one hand and the size of the animal and the number of removed arms on the other.

1. Taking up first the size correlation and using Fig. 3 as our basis of comparison, since it contains all the cases except those of Series V and therefore gives a more uniform and complete curve, we find that starting with the smaller individuals as we advance toward the larger ones there is a general increase up to a maximum at a diameter of 12 to 15 mm. This is most striking in the two later measurements, taken 33 days and 46 days after the operation. Thus in the 33-day measurement for Series I and II (Fig. 3), the regenerated length increases from 1.07 mm. for a disk diameter of 7 mm. to a maximum of 2.37 mm. for a disk diameter of 14 mm., and then goes down to .21 mm. for a 19 mm. diameter. Also for the Series III and IV at the same time the length increases from 2.04 mm. at a diameter of 7 mm. to a maximum of 3.45 mm. at a 12 mm. diameter, and down again to 1.36 mm. at a diameter of 18 mm. *The medium-sized individuals thus have the maximum rate of regeneration.*[1]

2. More striking still is the very constant difference between the regenerated lengths for Series I and those for Series IV in Fig. 2, and between the lengths for the combination of Series I and II and those for the combination of Series III and IV in Fig. 3. This shows a very decided advantage in favor of the animals with the greater number of removed arms. The difference is evident in the upper curves of Fig. 3 from measurements taken 22 days after the operation, but becomes more striking in the 33-day and 46-day curves. For example, in the 33-day curve for a 12 mm. diameter (the diameter at which we have the maximum rate of regeneration of Series III and IV) we get a regenerated length of 2.08 mm. for Series I and II, and of 3.45 mm. for Series

---

[1] Dr. Hans Przibram has called my attention to the fact that the specific rate of regeneration of the arms, *i. e.*, the amount of regeneration per unit of disk diameter as obtained from my data, does not show this increase from the smallest up to the medium-sized individuals, but gives a fairly constant figure up to 12 or 14 mm. The higher diameters then decline rapidly toward a minimum.

III and IV, an advantage of 1.37 mm. or 66 per cent in favor of the latter. Likewise, at a diameter of 14 mm. (where the Series I and II has its maximum regeneration) we get 2.37 mm. for Series I and II and 2.77 mm. for Series III and IV, an advantage of .4 mm. or 17 per cent in favor of Series III and IV. In a similar manner in the curves obtained from the 46-day measurements we get at a 12 mm. disk diameter a regenerated length of 2.46 mm. for Series I and II and 5.42 mm. for Series III and IV, and at a 15 mm. diameter 3.14 mm. for Series I and II and 3.72 mm. for Series III and IV, which represents an advantage for the group with the greater number of removed arms of respectively 2.96 mm. (= 120 per cent) and .58 mm. (= 18 per cent) for the two points named.

The difference between Series I and Series IV as represented in Fig. 2 is still greater. The regenerated lengths are on the whole at least twice as great in Series IV where four arms were removed as in Series I where only one arm was removed. Thus at a disk diameter of 8 mm. the regenerated length in Series I is 1.05 mm. and the average regenerated length in Series IV is 2.3 mm., an increase of 1.25 mm. or 119 per cent. For the same diameter at 33 days the respective values are 1.65 mm. and 4.4 mm., an increase of 2.75 mm. or 167 per cent. At 46 days the corresponding values are 2.0 mm. and 5.85 mm., an increase of 3.85 mm. or 192 per cent.

Likewise at a 12 mm. disk diameter for 22 days the values are .9 mm. and 2.1 mm., an advantage of 1.2 mm. or 133 per cent. At 33 days the values for a 12 mm. disk diameter are 2.3 mm. and 4.2 mm., an advantage of 1.9 mm. or 83 per cent, and at 46 days the corresponding values are 3.05 mm. and 6.5 mm., an advantage of 3.45 mm. or 113 per cent.

We may sum up the results on the rate of regeneration of the arms of the brittle-star, Ophioglypha lacertosa, as follows:

1. There is a definite relation between the size (*i. e.*, age [?]) of the animal and the rate of regeneration of its arms. The maximum rate is exhibited by individuals of medium size (with a disk diameter of 12 to 15 mm.). Both the smaller and the larger ones give a diminishing rate as we go away from this point.
2. The greater the number of removed arms (excepting the case where all are removed) the greater is the rate of regeneration of each arm.

4. *Discussion.*

We must, therefore, conclude that when more than one arm is removed the regenerative energy as expressed in the replacement of the lost arms is greatly increased. Not only is the total regenerative energy greater in this case, but the energy expressed in each arm is greater than the total energy when only one arm is removed.

Expressing this in mathematical form, if E_1 represents the regenerative energy exhibited in the replacement of the lost arm when only one is removed, assuming that increase in length is a measure of such energy, and E_n represents the energy exhibited in regeneration when more than one arm is removed, n being the number of absent arms, then not only is $E_n > E_1$ but also

$$\frac{E_n}{n} > E_1 \text{ or } E_n > n\, E_1.$$

Therefore, when we remove n arms we increase the total regenerative energy by more than n times the amount exhibited when only one is removed. The force of this statement is made especially strong when we consider that throughout the experiments the animals received no food supply whatever.

Expressing the relation in still another way, let us take a brittle-star with arms A, B, C, D and E, in which a_1, b_1, c_1, d_1 and e_1 represent the respective lengths these arms will attain after a definite period of regeneration, supposing that one alone is cut off in each case. Now let us suppose instead that the first four are cut off, then after this same period of time we get for the regenerated lengths $a_4 > a_1$, $b_4 > b_1$, $c_4 > c_1$, $d_4 > d_1$. Now in the first case we cannot assume that the stimulus of removal and the resultant reaction of regeneration are purely local and concern only the tissues in the immediate vicinity of the cut surface, for we then get into difficulty as soon as we try to explain the cases where four arms are simultaneously removed. Here we find we must add a considerable quantity (r_4) to each of the original single regeneration lengths to get the new regeneration length, *e. g.*, $a_4 = a_1 + r_4$. Then $a_4 + b_4 + c_4 + d_4 = a_1 + b_1 + c_1 + d_1 + R_4$ where R_4 $(= \Sigma\, r_4)$ represents the total response of the organism as a whole which must be added to the local effects of the operation stimulus. If, on the other hand, we consider the influence of the organism as a whole on the regeneration of its arms as one of retardation, we must take the values a_4, b_4, c_4 and d_4 as representing most nearly the original local stimulus effect. Then without changing the values of r_4 or R_4 we may rearrange the formulæ, making $a_1 = a_4 - r_4$, etc., and $a_1+b_1+c_1+d_1 = a_4+b_4+c_4+d_4-R_4$.

The regeneration of the arms of Ophioglypha thus offers us a very good example of the influence of conditions away from an injured surface upon the regeneration at that surface. The result may be stated in two ways and each mode of statement may be made to lead to a separate mode of interpretation.

We may say that the rate of regeneration increases with an increase in the number of removed arms. With this statement as a starting point it is natural to assume that, in the cases where more than one arm is removed, the stimulus of the additional operations or of the additional regenerating organs exerts an accelerating influence upon the regenerating tissues at any one such surface.

Another mode of statement is the following: The increase in the number of removed arms is necessarily accompanied by a decrease in the number of uninjured arms present, and *the rate of regeneration of a removed arm therefore increases as the number of uninjured arms still remaining decreases.* If the uninjured arms exert a retarding influence upon the regenerating tissue at an injured surface we can understand why a removal of additional arms may bring about an increase in rate of regeneration of each. The discussion of this interpretation involves the whole problem of nutrition and perhaps the whole general problem of form regulation as well. It will be best to reserve further discussion until we have examined the other experiments to be described in the following pages.

But whether we consider the influence of the organism as a whole to be one of acceleration or one of retardation, we must recognize in either case that the regeneration rate is not a matter which involves only the local conditions at the wounded surface as determined by the direct action of the operation. It seems, on the other hand, to be bound up with intricate reactions affecting the whole character of the activities and organization of the animal.

III. THE OPERCULA OF SERPULIDS.

We have now considered a case (section one) in which there is a readjustment in the uninjured portions of a system as a result of their mutual interaction. This interaction is not complicated by the addition of a regenerating organ. The result is a new system in equilibrium, based on the resultant of the interactions of the uninjured parts.

In a second section (section two) a case was considered in which it was possible to study the effect of the presence or absence of uninjured portions of the animal upon the rate of regeneration of the removed ones. The reaction is here more complicated than in the first case, because there may be here an action of other regenerating surfaces upon any particular wounded surface as well as the action of the uninjured organs themselves.

In the present section (section three) a case will be considered in which there are two organs, dissimilar in size, situated on morphologically similar opposite sides of the median line. An extremely close interaction is found to exist between these two organs so that any disturbance in one is reflected in changes in the other. This close interrelation between the opercula of the Serpulids, the organs in question, gives a good basis for the study of such interactions as were outlined in the general introduction. The opercula of the Serpulids furthermore furnish exceptionally good material for this study of compensatory regulation because of the various degrees of asymmetry present in the different species.

In the following account it will be necessary to go into paths not in the line of the main discussion, but such a course cannot be avoided in a study of the factors controlling the regulation of the opercula.

In the adult Serpulids of the genus Hydroides we have an asymmetrical stable system with the functional operculum on the right side and the rudimentary operculum on the left or vice versa. The nature of this case will first be taken up. Then the opercula of other members of the family will be described. This will be followed in turn by a description of the ontogenetic development, the regeneratory development, some speculations as to the probable phylogenetic development, a discussion on the comparison of regeneratory, ontogenetic and probable phylogenetic development and finally by a discussion of the facts of compensatory regulation as here exhibited.

In a separate section (section four) a special series of experiments on the regulation of the rate of differentiation of the opercula in the Serpulid, Apomatus ampullifera, will be treated.

1. *Comparative Anatomy.*

1. The Genus Hydroides.

The opercula and branchiæ of the genus Hydroides will serve as the type in our description of the anatomy of these structures throughout the family. The branchiæ are brought in only incidentally as our main purpose is to get the details of the structure of the opercula to serve as a basis for the regeneration and regulation experiments to be described later. Unless otherwise stated the description applies to H. dianthus. H. uncinata and H. pectinata, living in the Mediterranean at Naples, were also used in the experiments and the differences will be pointed out at the close of the special description. H. dianthus is found on the Atlantic coast of North America living attached to stones, mollusk shells and other hard materials, from low water mark to a depth of several fathoms. The worm lives in an irregularly twisted calcareous tube which is attached by its side to the supporting surface. The tube increases in size from the posterior end anteriorly and is continually being built up at the anterior end by additions from the special calcareous glands.

The body of the animal is very distinctly divided into the thorax and the abdomen. The first-named region is marked by the presence of the broad, flat fold of the thoracic membrane, which is continued at both the anterior-ventral and the posterior-ventral ends as a projecting membrane. At the anterior end this membrane forms a collar which, except for a slight break on the dorsal side, completely surrounds the head end. (See Fig. 5A.)

Upon the anterior surface thus enclosed by the collar are located the two semicircular rows of branchiæ, one on each side of the mouth, which apparently serve on the one hand as organs of respiration and on the other through their cilia as agents for the creation of a current of water carrying food particles to the mouth. The two rows of branchiæ are placed on slight ridge-like elevations, the branchial ridges. These are not strictly semicircular in shape but the ends of each are curved inward. This shape evidently has some connection with the proper collection of the food particles carried downward by the cilia. (See Fig. 5B, C.) The number of the branchiæ increases with the age of the animal and in fully grown individuals there are about fourteen on each side.

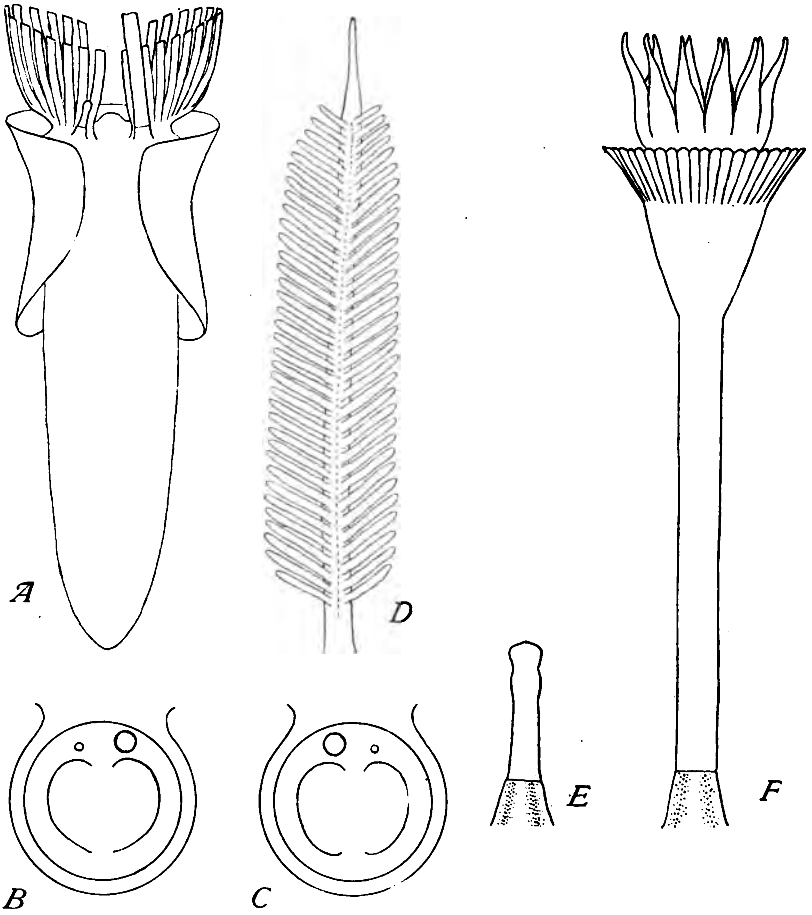


Fig. 5.

Hydroides dianthus. *A*—Dorsal view of right-handed specimen, showing relations of parts. Ends of branchiæ and functional operculum not given (× 6). *B, C*—Diagram of anterior surface of head of left-handed and right-handed specimens (× 6). *D*—Branchia viewed from inner surface (× 25). *E*—Rudimentary operculum (× 30). *F*—Functional operculum (× 30).

Each branchia consists of a long axis bearing two rows of secondary processes, the pinnules. (See Fig. 5D.) The axis is continued for a short distance beyond the region of the secondary processes as a slender tapering thread. The pinnule rows slope inward so as to inclose a trough-like area, V-shaped in cross section and with the cavity of the trough pointing inward, *i. e.*, toward the mouth. The surfaces bordering this area are ciliated and it is along them that the food-bearing currents are formed.

Near the dorsal end of one of the branchial ridges, not in the line of the branchiæ but dorsal to it, there is a stout, naked stalk of approximately the same length as a branchia but bearing at its distal end a funnel-shaped expansion. (See Fig. 5F.) The whole organ constitutes the functional operculum. The edge of the expanded portion is marked by teeth-like serrations, the hollows between which are continued for some distance down the outside. From the center of the terminal circular area within this row of serrations there arises a group of secondary processes, arranged so as to form a cup-shaped figure. The ends of the processes are usually hooked and considerable foreign material often clings to them. The whole organ serves as a very efficient plug for the open end of the tube when the animal has retired within for protection. An examination of the place of attachment of the opercular stalk shows that it is located dorsal to the first branchia or sometimes nearly opposite the interval between the first and second branchiæ. Near the base of the stalk there is a transverse suture varying in distinctness in different cases and which, as we shall see later, is a "breaking joint," an important structure in the experiments.

On the opposite side of the mid-dorsal line, and in a position corresponding in all respects with that of the large operculum, is a small organ consisting of a slender stalk with a slight terminal enlargement. (Fig. 5E.) It also shows a distinct line of demarcation between a darker colored more basal region and the lighter remainder of its body. This small organ, the "pseudopercule" of de St. Joseph is most commonly called the rudimentary operculum.

A study of the relative positions of the opercula is interesting. An examination of 244 adult individuals of H. dianthus gave 139 or 57 per cent with the functional operculum on the right side and 105 or 43 per cent with it on the left. The distribution between right and left is thus fairly equal though there is a considerable advantage in favor of those with the functional operculum on the right side and the rudimentary on the left. Similarly in H. uncinata out of 16 specimens ten had the functional operculum on the right side and six on the left, and in H. pectinata out of 41 specimens 21 were right handed and 20 left handed.

An examination of the internal structure of the branchiæ and opercula brings out a close agreement between the two in anatomical details. Their morphological agreement has been especially emphasized by Örley and Meyer. Örley ('84) compares the internal anatomy of the branchia and the functional operculum in Serpula. He makes no mention of the rudimentary operculum. According to him an operculum corresponds morphologically with a branchial stalk, all the pinnules of which have been collected at the end in one bundle. He describes the presence of an axial blood vessel in both branchial and opercular stalks. In the branchial stalk, however, he saw only one nerve trunk (the axial one) while in the opercular stalk two lateral ones were shown. Meyer ('88) showed the more complete similarity of the branchia and operculum in Eupomatus uncinata (= Hydroides uncinata), while at the same time pointing out the incompleteness of Örley's observations and the error in his mode of homology. He describes three nerve trunks in both branchiæ and operculum, although the middle one is very small in the opercular stalk and does not reach much more than halfway to the distal end. In the branchia the two lateral ones likewise are very insignificant. Meyer points out that this difference is probably due to the fact that the pinnules and ciliated groove are innervated from the middle nerve, so that this has a greater development in the branchiæ where pinnules and ciliated groove are present than in the opercular stalk where they are absent. The stalk of the operculum is thus made directly homologous with a branchial axis lacking its pinnules.

A study of the internal structure of the branchiæ and opercula of Hydroides dianthus brings out points which are in entire agreement with the conclusions of Meyer and which emphasize the close similarity of the branchiæ and opercula.

An interesting characteristic is further made out in the functional operculum. There is a difference between the cells near the basal region and those in the middle and terminal regions of the stalk. Near the base of the stalk in the region below the "breaking joint" the cells of the connective tissue have more of an embryonic character than elsewhere, having fewer and shorter processes and less intercellular material. This distinction has already been noted by Orley ('84) in his description of the connective tissue of the opercular stalk in Serpula vermicularis. He says, speaking of this tissue, "Die Modificationen dieses Bindegewebes sind nach den Ortsverhältnissen sehr verschieden. *Im innersten Theile des Stieles* wo dieser mit dem Kiemenlappen zusammen hängt, findet man kleine weniger verzweigte Zellen in der sehr spärlichen Intercellularsubstanz. *Es ähnelt sehr der embryonaler Form.* Etwas höher trifft man bereits Zellen an, die sich durch Grösze und durch die Zahl ihrer Ausläufe auszeichen und eine gut entwickelte Intercellularsubstanz haben."[1] The significance of the differences of the regions will be brought out in connection with the experiments described later in the paper (p. 55).

The rudimentary operculum of H. dianthus has two well-defined regions. The cells distal to the "breaking joint" are distinctly embryonic in form and general character. Those proximal to the breaking joint have among them well-developed supporting cells of the type found in the branchiæ and functional operculum, though these cells are not as highly differentiated as in the latter organs.

A comparison of the two other members of the genus Hydroides with H. dianthus brings out only slight differences in the character of the opercula and branchiæ. H. pectinata, however, has pectinate secondary processes as opposed to the unbranched ones of H. uncinata and H. dianthus.

Any conclusion drawn from direct anatomical evidence must emphasize a very close resemblance in the internal structure as well as in the position of the opercula and branchiæ. A similar conclusion as regards the morphological worth of the rudimentary operculum can be reached by a recognition of similarity in position on the one hand and the nearly equal appearance of right and left-handed individuals on the other.

The functional operculum in Hydroides is therefore morphologically a branchia which has formed an expansion at its distal end and which has at the same time lost its respiratory pinnules.

---

[1] Italics mine.

The increase in strength of the supporting axis is a necessary concomitant of the other changes.

The rudimentary operculum cannot be compared directly with a branchia because of its bud-like appearance and embryonic tissues. In position it, however, corresponds perfectly with the functional one and therefore with the branchiæ as well.

2. Other Genera of Serpulids.

An examination of the different groups of Serpulids brings out the fact that we have almost all gradations between forms with no opercular modification of the branchiæ and forms with the single operculum possessing scarcely any trace of a branchial character.

a. Group I. No Opercular Differentiation. Examples of Serpulids with no opercular modification of the branchiæ are Protula (Risso) and Protis (Ehlers). Each branchia possesses respiratory pinnules and tapers to a point at its distal end. The branchiæ resemble one another throughout both right and left circlets. The members of this group are able to retreat for a long distance back into the tube, in this respect resembling the Sabellids which also have no opercula. (See Fig. 6A.)

b. Group II. Each Branchia with a Terminal Enlargement. In Salmacina Dysteri Huxl. there are eight branchiæ, four on each side and each has a terminal club-shaped enlargement. The branchial stalk or axis has from fifteen to twenty pairs of ciliated pinnules. The two rows of pinnules are bordered on the outside by enlarged mucous cells which near the distal end spread out along the sides of the club. This enlarged region bears no pinnules. The eight branchiæ are similar in their characters. It is evident that when the animal retreats into its tube these enlarged ends must collectively serve as a stopper for the opening and thus barricade the end more effectively than those of Protula which bear no such enlargements (Fig. 6B).[1]

c. Group III. Two Equal Opercula, Right and Left, on Ends of Branchiæ. Branchial Pinnules Present. Filograna implexa resembles Salmacina in having eight branchiæ. The dorsal one on each side is, however, terminated by a small, transparent, chitinous, spoon-shaped structure obliquely attached to the side of the tip of the axis of the branchia. The other branchiæ end in short blunt points. The two opercula are equal in size and the stalks which bear them retain the pinnules and other branchial characters (Fig. 6c). When the animal has withdrawn into its tube the branchiæ are twisted in spiral form and the two opercula are superimposed, the one upon the other. The more anterior one closes the tube after the manner of forms with but one operculum. The more posterior operculum, therefore, serves as a protection only in the cases where the barricade formed by the first is not effective. As compared with Salmacina, to which it is otherwise closely related, Filograna has two, more effective oper-

---

[1] de St. Joseph, however, seems unwilling to admit an opercular function for these structures.

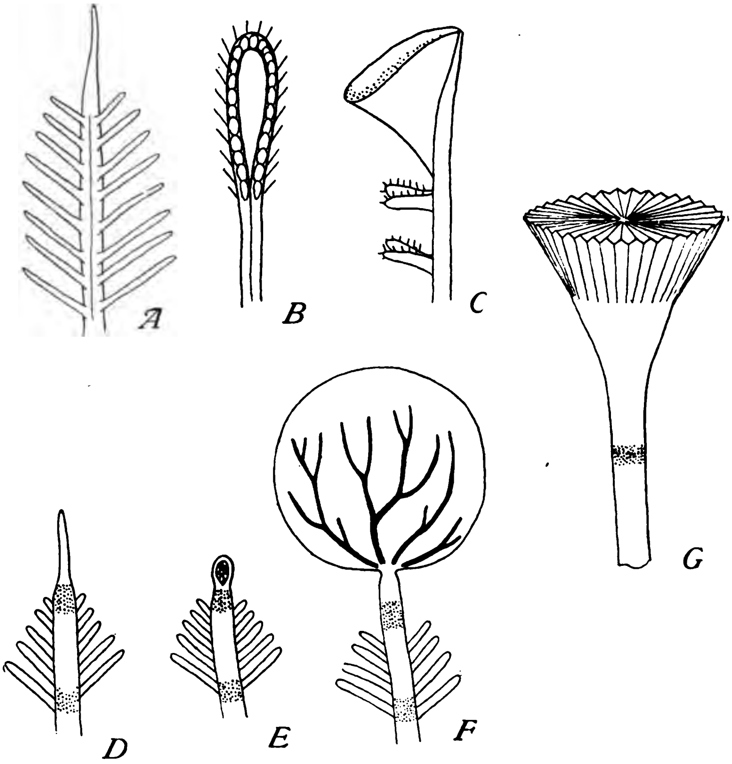


Fig. 6.

A—End of branchia of Protula. *B*—Club-shaped end of branchia of Salmacina (after de St. Joseph). *C*—One of the two opercula of Filograna (after de St. Joseph). *D*—Tip of non-operculate branchia of Apomatus ampullifera (× 17). *E*, *F*—Tip of rudimentary operculum (*E*) and functional operculum (*F*) of same (× 17). *G*—Distal portion of functional operculum of Serpula vermicularis (× 19).

cula instead of eight less effective club-shaped enlargements. The fact that when the animal is retracted the expanded portion of one operculum occupies a position in front of the other may be of importance in connection with a theory of the development of asymmetry in these organs in other members of the group.

d. Group IV. One Functional Operculum and One Rudimentary Operculum. Both on ends of Branchiæ. Pinnules present. Examples—Apomatus, Josephella.

In Apomatus the next to the dorsal branchia on either the right or the left side is expanded at its end into a globular almost transparent operculum. The chitinous shell of the sphere itself contains irregularly branched blood vessels, the green-colored blood of which makes them very conspicuous. The branchia in a corresponding position on the opposite side has a small ovoid enlargement with a very pronounced network of blood vessels containing distinctly pulsating green blood. Both these opercula are placed at the ends of stalks which retain all the branchial characters in an unchanged condition.[1] (Fig. 6D, E, F.)

In a few cases the branchia occupying the place of the rudimentary operculum ended in a tapering point instead of an ovoid enlargement. There are usually about twenty pairs of branchiæ in the adult. Each of the two circlets breaks off very readily along a definite breaking plane level with the anterior surface of the head. The division plane is very pronounced and the break is clean cut and takes place so readily that it is very hard to remove the animal from its tube without causing it to throw off both of the branchial circlets.

e. Group V. One Functional Operculum and One Rudimentary Operculum. Functional Operculum with Naked Stalk. Rudimentary Operculum Not on End of Long Stalk. Examples—Serpula, Crucigera, Hydroides.

The description given above for Hydroides (p. 21) is sufficient as a general characterization of this type. The functional operculum may be either on the right or on the left side, the rudimentary operculum in each case occupying the opposite position. The opercula are not in the line of the branchiæ but occupy a position dorsal to the first dorsal branchiæ or to the interval between the first and second dorsal ones. Serpula differs from Hydroides in the entire absence of the secondary group of processes in the operculum (Fig. 6G). Crucigera has only four secondary processes and these are arranged in the form of a cross (de St. Joseph, '94).

---

[1] According to de St. Joseph ('94) the functional operculum appears on the left side and the rudimentary, his "pseudopercule," on the right. He does not mention the possibility of the reverse arrangement. The specimens which I examined at Naples showed a preponderance of the right-handed condition. (See p. 32.)

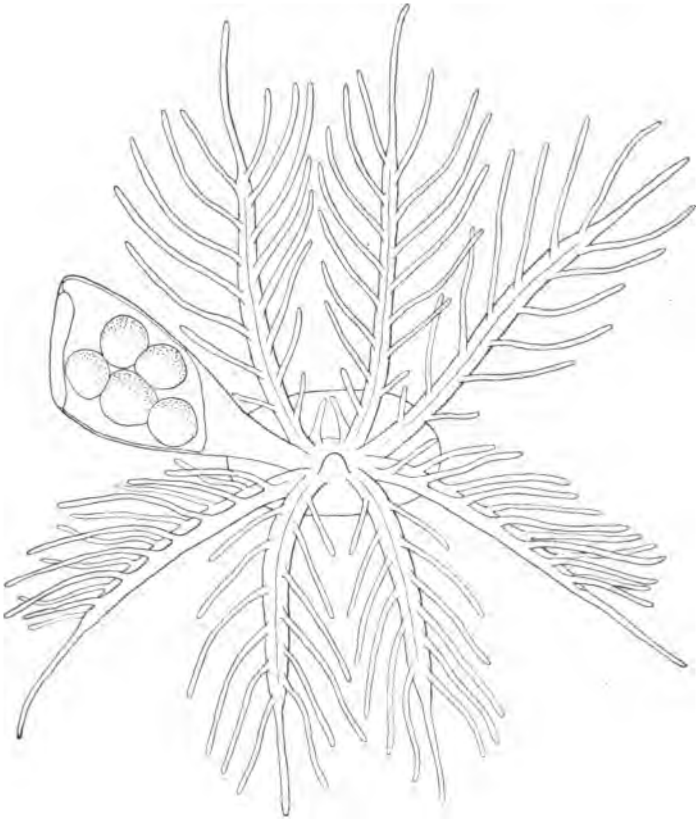


Fig. 7.

Spirorbis Pagenstecheri. Ventral (slightly anterior) view showing branchiæ and operculum with its brood chamber containing embryos (×40).

f. Group VI. One Operculum. No Rudimentary Operculum. The members of this group have only one operculum. There is no rudimentary operculum. Examples are Spirorbis, Pileolaria, Ditrupa, Filogranula (?), Pomatoceros, Vermilia.

This group may be further subdivided according as the operculum has a position in the line with the branchiæ (Ditrupa,

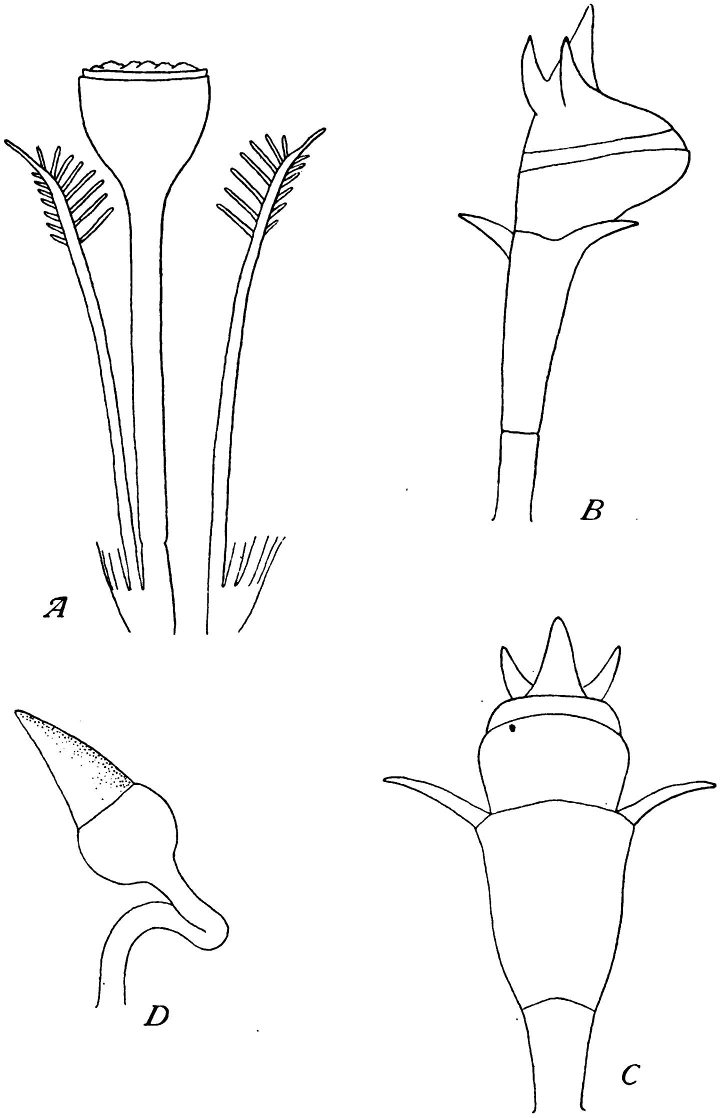


FIG. 8.

A—Ditrupa subulata, showing single naked stalked operculum in line with branchiæ of left side. Dorsal view. Note indication of basal suture (× 15). *B*, *C*—Side view and dorsal view of operculum of Pomatoceros triquetroides, showing highly modified terminal and lateral spines. Basal suture present (× 10). *D*—Operculum of Vermilia multivaricosa, showing curved stalk and absence of a basal suture. Dorsal view—Left-handed individual (× 8).

Spirorbis, Pileolaria, Filogranula [?]) or dorsal to the line of the branchiæ (Pomatoceros, Vermilia and others). In the latter case the operculum may further be either at one side of the median line (most species of Pomatoceros and Vermilia) or in the middle line itself (Pomatoceros elaphus, Haswell).

In Ditrupa there is a large cup-shaped operculum with a naked stalk situated on the left side in line with the branchiæ. It occupies a position on the median side of the most dorsal branchia of that side (Fig. 8A). I had only four specimens for examination. In all of these the operculum was on the left side, but whether this was merely a coincidence or not it is impossible to say. The tube of Ditrupa subulata lies freely on the sea bottom usually at a considerable depth. Its substance is extremely hard and difficult to break. The tube is slightly curved and resembles very much the shell of the mollusk Dentalium.

The Spirorbis-like forms (Spirorbis, Pileolaria, etc.) have closely coiled tubes attached by the dorsal side to a flat surface. In some the tube is attached for its whole length but in others (some species of Pileolaria) the end may rise up from the level of the surrounding surface. The direction of the coil of the tube is constant for any one species but varies in the different species. Thus dextral and sinstral species are distinguished according as the tube is coiled clockwise or counter clockwise. The dorsal side of the animal is next to the attached surface and the posterior end of the animal upon removal has a pronounced curve to the right or left according as the tube is dextral or sinstral. In the dextral species the operculum is on the right side and in the sinstral on the left so that in all cases the operculum is on the side next to the concave curve of the shell. The number of branchiæ varies in the different species from five to twelve, according to Caullery and Mesnil ('96). Of the two species examined by me Spirorbis Pagenstecheri had four branchiæ on the left side and three plus the operculum on the right and Pileolaria sp. had five branchiæ on the right and four plus the operculum on the left. In both cases the operculum occupies the position of the next to the dorsal branchia on its side, *i. e.*, the right side in Spirorbis and the left side in Pileolaria. In all members of the group the operculum is in line with the branchiæ. It is as a rule much smaller than the opening of the tube so that the animal can retreat to a considerable distance within the tube. There is no sign in either of the two genera of any modification of the branchiæ to compare with the rudimentary operculum of Apomatus or Hydroides. The right or left position of the operculum is definitely correlated with the direction of curvature of the tube and as the latter is constant for any one species the former must be also. In Spirorbis Pagenstecheri the operculum serves as a brood pouch and is of a trumpet shape. The branchial pinnules are comparatively large and it is sometimes hard to tell whether a basal pinnule should or should not represent a branchial stalk. (Fig. 7.) Pileolaria sp. also uses its operculum as a brood pouch. Other species, however (S. borealis, for example, according to Alex. Agassiz, '66), keep the eggs in a string within the tube on the ventral side of the body.

Next comes the group in which the single operculum does not occupy the line of the branchiæ but is dorsal to it. First are the cases in which it is lateral. In Pomatoceros triquetroides the operculum is very large and stout. There are two lateral processes beyond which comes an expansion ending in a cap of three spines. There is a very pronounced suture near the base. The whole region below the two lateral processes is flattened dorso-ventrally. In the cases examined the operculum was always on the left side (Fig. 8B, C).

In Vermilia multivaricosa the operculum occupies a position corresponding with that of P. triquetroides but it may be either on the left or on the right side. The stalk of the operculum is approximately circular in cross section though possessing a corrugated outer surface. It loops around from its point of attachment toward the median line. The terminal region is thus brought nearer to the median line than is the proximal region. The terminal portion is very large and heavy. There is a basal globular portion upon which rests a heavy cone-shaped body (Fig. 8D).

Haswell ('85) describes a species of Pomatoceros (P. elaphus) with a large median operculum which is short and flattened dorso-ventrally. At the sides of the proximal portion are two wing-like lobes bearing small processes. Terminally there are three processes with antler-like branches. In another Serpulid (Vermilia cæspitosa), according to Haswell, there is also a large operculum on a short stubby stalk (but not median judging by the figure, though there is no statement in the paper on this point). This operculum is armed terminally with peculiar spines and serrated processes and also has two lateral wings like those of Pomatoceros elaphus but not as well developed as the latter. E. Meyer ('88) concludes that these opercula have been formed by the union of two lateral ones, but the evidence as regards this point is by no means conclusive, since we have species where similar opercula are evidently lateral in position (P. triquetroides and V. multivaricosa, for example).

g. Summary. The principal modifications of the opercula throughout the family of Serpulids have now been passed over briefly and the general characters may be summarized. In the *first* group are the forms with no opercular modification (Protula, Protis). In the *second* each branchia has a small club-shaped enlargement, the combination of the enlarged ends no doubt making a more or less effective barricade against invaders when the animal has retreated into its tube (Salmacina). In the *third* group (Filograna) the modification is confined to the most dorsal branchia on each side. All the others lack opercular differentiations. The two dorsal ones mentioned also retain their branchial characters, but in addition each has an operculum of sufficient size to close up the opening of the tube. In the *fourth* group (Apomatus, Josephella) there is one functional and one rudimentary operculum, one on the end of each of the two next to the dorsal branchiæ. The stalks supporting these opercula retain their branchial pinnules and other branchial characters. In the *fifth* group (Serpula, Crucigera, Hydroides) there are likewise one functional and one rudimentary operculum, the distribution between right-handed and left-handed forms being fairly equal in adults. The opercula, however, do not possess branchial pinnules though their internal anatomy and position indicate branchial characters. The rudimentary operculum is not situated on the end of a long stalk but is a small bud-shaped organ corresponding in position with the functional operculum. Judging by their position the opercula seem to have moved down from the interval between the most dorsal and the next to the dorsal branchiæ on each side. Finally in the *sixth* group there is only one operculum and this retains but little of its branchial character. In some of the group, however (Ditrupa, Spirorbis, Pileolaria), it retains its position in the branchial circlet. In some cases it may be used as a brood pouch (Spirorbis, Pileolaria). In other forms (Pomatoceros, Vermilia) it is a considerable distance below the branchial region and is large and massive. In some of the species as P. triquetroides and V. multivaricosa it has a lateral position and in others, as P. elaphus, a median one.

These six groups form a very complete morphological series which points strikingly toward the homology of the opercula and branchiæ in all the forms.

3. Distribution of the Opercula between Right and Left Sides.

The data upon this point will be presented under this separate heading because of their special interest in connection with later discussions. The first three of the groups of Serpulids mentioned above exhibit no asymmetry in their opercula and need not be considered here. The three others will be discussed in turn:

a. The *fourth group* have one functional and one rudimentary operculum. Both opercular stalks have branchial pinnules. (Examples—Apomatus, Josephella.)

b. The *fifth group* have one functional and one rudimentary operculum, each with a naked stalk. Rudimentary operculum not on end of a long stalk. (Examples—Serpula, Crucigera, Hydroides.)

c. The *sixth group* have only one operculum. No rudimentary operculum is present. (Examples—Ditrupa, Spirorbis, Pileolaria, Pomatoceros, Vermilia.)

(*a.*) In the *fourth* group thirteen specimens of Apomatus were examined and of these *ten* had the functional operculum on the right side and the rudimentary on the left. The other three had the reverse condition with the functional on the left and the rudimentary on the right.

Table II.

	Total No.	F = Right R = Left	F = Left R = Right
Apomatus ampullifera....................	13	10	3
Per cent	—	77	23

(*b.*) In the *fifth* group Hydroides dianthus, H. uncinata, H. pectinata and one specimen of Serpula vermicularis were examined for the distribution of opercula. By far the most extensive observations are on Hydroides dianthus.

TABLE III. *Hydroides dianthus. Position of Opercula.*

Locality and Date.	Total No.	F = Right. R = Left.	F = Left. R = Right.	Not like other two groups.
Woods Hole, Mass. .... 1901/IX/16–IX/19.	57	31	24	2*
Cold Spring Harbor, L. I.... 1902/VII/5–6–7.	74	39	30	5†
Cold Spring Harbor, L. I.... 1902/VIII/9 to 20.	120 + ?7‡	69	51	?7‡
	251 + ?7			7 + ?7
Total.................	=258	139	105	=14
		53·9%	40·7%	5·4%

EXPLANATION OF TABLE.

F = Functional operculum; R = Rudimentary operculum.

*These two specimens had a rudimentary operculum on each side.

†These irregulars come under four heads: 1. *One* specimen with Right = operculum missing; Left = operculum between rudimentary and functional stage. 2. *One* specimen with Right = between rudimentary and functional; Left = rudimentary operculum. 3. *Two* specimens with Right = functional operculum; Left = two-thirds developed functional. 4. *One* specimen with Right = rudimentary; Left = one-half developed functional.

‡In this case the number of unclassified irregular cases was unfortunately not put down. My notes give only the indefinite statement "several abnormal and incomplete ones observed are not included in the present list." Assuming that the percentage of such cases is the same as in the other two groups, where it is 5.4 per cent of the total number, we will not be far astray in making the unknown number equal to seven.

The relative relation between right-handed and left-handed forms is expressed to better advantage if the irregular cases are not included. Removing the last column from the former table we get the relations expressed in the following one:

An examination of this table shows that 57 per cent of the "normal" cases have the functional operculum on the right and the rudimentary on the left, while 43 per cent have the opposite arrangement. The striking agreement in the three sets of figures, one from Woods Hole and the other two from Cold Spring Harbor, indicates the probability of some organic basis back of the fact. This matter will come up again later in the discussion of the development in ontogeny and during regeneration. At the same time the considerable number of cases (fourteen [?] or 5.4 per cent of the whole number) which do not come under either of these groups will be taken up.

TABLE IV. *Hydroides dianthus. Position of Opercula (not including irregular ones).*

Locality and Date		F = Right R = Left	F = Left R = Right	Total
Woods Hole, Mass.,	No.	31	24	55
1901/IX/16–19	Per cent	56.4	43.6	—
Cold Spring Harbor, L. I.,	No.	39	30	69
1902/VII/5-6-7	Per cent	56.5	43.5	—
Cold Spring Harbor, L. I.,	No.	69	51	120
1902/VIII/9–20	Per cent	57.5	42.5	—
	No.	139	105	244
Total	Per cent	57	43	—

TABLE V. *Hydroides uncinata. Position of Opercula.*

Locality and Date		F = Right R = Left	F = Left R = Right	Total
	No.	10	6	16
Naples, 1902/XI/21	Per cent	62.5	37.5	—

Sixteen specimens of Hydroides uncinata were examined at Naples. Of these ten, or 62.5 per cent, had the functional operculum on the right and the rudimentary on the left, and six, or 37.5 per cent, had the reciprocal arrangement; a considerable advantage in favor of the right-handed ones.

Likewise 41 specimens of Hydroides pectinata were examined at Naples, and of these 21 or 51.2 per cent had the functional operculum on the right side and the rudimentary on the left while 48.8 per cent had the reciprocal arrangement, a slight advantage in favor of the right-handed ones.

TABLE VI. *Hydroides pectinata. Position of Opercula.*

Locality and Date		F = Right R = Left	F = Left R = Right	Total
Naples, 1902/IX/27	No.	21	20	41
1903/III/31	Per cent	51.2	48.8	—

In Serpula vermicularis I examined only one specimen and this had the functional operculum on the left side and the rudimentary on the right.

Our general conclusion regarding the distribution of the opercula in the *fifth* group of Serpulids, those with the naked-stalked functional and small bud-like rudimentary is that the right and left-handed forms are nearly equal in number, but there is a slight advantage in favor of the right-handed ones.

(*c*.) In the *sixth* group there is only one operculum.

In Ditrupa subulata only four specimens were examined as regards this point. All four of these had the operculum on the left side.[1]

In the dextrally coiled Spirorbis Pagenstecheri *eleven specimens were examined* and all had the operculum on the right side, while in the sinstrally coiled Pileolaria the one specimen examined had the operculum on the left. The observations of Caullery and Mesnil ('96) show that in the species with dextrally coiled tubes the operculum is always on the right side and in the sinstrally-coiled ones always on the left side.

---

[1] de St. Joseph ('98) gives a description of Ditrupa arietina, O. F. Müller (= D. subulata, Desh.) in which he mentions the operculum as a structure of the left side in agreement with the present observations. Also the left side according to him has one less branchia than the right, *i. e.*, there are 11 branchiæ (plus the operculum) on the left and 12 on the right.

In Pomatoceros triquetroides 21 specimens were examined and all had the operculum on the left side. This makes a fairly strong probability in favor of the permanence of such a characteristic. As a further argument may be mentioned the statement of de St. Joseph ('94), who mentions the observation of Grube on 63 specimens of P. triqueter, L.(= P. triquetroides, D. Ch.) and of himself on many more than this number which showed the operculum in every case on the left side, so that we may be fairly certain that in this species the organ is a permanent structure of the left side.

In Vermilia multivaricosa eleven specimens were examined. *Six* had the operculum on the right and *five* on the left side. In this case, therefore, there seems to be a fairly equal distribution between the two sides.

The data for Group VI are collected in the following table:[1]

TABLE VII. *Group Six. Position of Operculum. Locality: Bay of Naples.*

Name and Date.	No.	Operc. Right.	Operc. Left.	Per cent Right.	Per cent Left.
Ditrupa subulata 1903/I/10.	4	0	4	0	100
Spirorbis Pagenstecheri 1903/I to V.	11	11	0	100	0
Pileolaria sp. (?) 1903/III/2.	1	0	1	0	100
Pomatoceros triquetroides 1903/I/25, IV/3, IV/5.	22	0	22	0	100
Vermilia multivaricosa 1903/I/16, III/5.	11	6	5	55	45

Discussion of the Evidence from Group VI. In Spirorbis Pagenstecheri and Pileolaria sp. the position of the operculum bears a direct relation to the direction of the coil of the tube. Caullery and Mesnil ('96) have shown that the operculum of Spirorbis and its relatives is always located on the side next to the concave surface of the coil, *i. e.*, on the right hand side in dextral tubes and on the left-hand side in sinistral ones. Whether or not any such relation can be made out in other members of Group VI it is not possible to say. In Ditrupa there is a slight curvature of a definite form in the tube but the relation of the body within the tube has not been made out definitely enough to determine the significance of the constancy of position of the operculum. In Pomatoceros triquetroides there is a definitely fixed left-handedness though the tube is irregularly coiled, and in Vermilia multivaricosa there is an almost equal distribution between the two sides, the tube being again irregularly coiled.

---

[1] Former observations on the position of the operculum in this group are those of Caullery and Mesnil ('96), who state that dextrally coiled Spirorbis-like Serpulids always have the operculum on the right side (example—Spirorbis Pagenstecheri) while sinistrally coiled ones have it on the left side (example—Pileolaria sp. [?]). de St. Joseph ('98) describes the operculum of Ditrupa subulata, Desh., as a structure of the left side. de St. Joseph ('94) for himself and also for Grube describes Pomatoceros triquetroides as always left-handed.

A discussion of the factors controlling the determination of the position of the opercula must be left until the ontogenetic and regeneratory development of these organs have been studied.

4. Exceptional Degrees of Development in General and One Case of a Supernumerary Operculum.

The two opercula in Groups IV and V in the vast majority of cases consist of a fairly typical functional and a fairly typical rudimentary operculum. There are, however, exceptions to this statement, as has already been indicated above. The main exceptions are due to the partial further development of what probably would otherwise correspond with the rudimentary operculum, either with or without the loss of the functional operculum. There thus arise either one functional operculum and one partly developed functional or a missing operculum and one partly developed one. In other individuals both opercula were found to be rudimentary or one rudimentary and one partly developed one were present. The discussion of these cases must be reserved until we come to the experimental part of the paper.

One case of a supernumerary operculum was found and this is interesting in connection with the problem of the regulation of the opercular development. The accompanying figure (Fig. 9) gives the relations of the opercula. On the left-hand side there is a rudimentary operculum of the typical form in the usual position. On the right-hand side in a corresponding position is a typical functional operculum, but in addition to it there is an added rudimentary operculum with its point of attachment posterior to that of the functional. This added rudimentary operculum agrees in all respects with the one on the opposite side, so that we have two rudimentary opercula and one functional one. The specimen indicates that the influence which determines the development of a functional or a rudimentary operculum is not always a bilaterally differentiated one. A more complete discussion must be reserved until the experimental data have been given.

2. *Development of Opercula.*

1. Ontogenetic Development.

a. Introduction. In a paper, entitled "A Case of Compensatory Regulation in the Regeneration of Hydroides Dianthus," I described some experiments showing that when the functional operculum of this Serpulid is removed the rudimentary operculum on the opposite side develops into a new functional operculum similar to the old one while in place of the old functional stalk a new rudimentary bud develops. In the discussion of this and similar experiments it was stated that a knowledge of the ontogenetic development of the organ is highly desirable before we can be in a position to discuss the data in their full relations. With this object in view the writer undertook to raise the larva up to the stage where both opercula have attained their normal adult development.

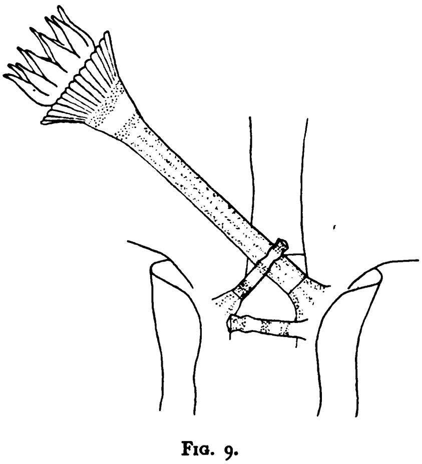


Fig. 9.

Hydroides dianthus with three opercula, two rudimentary and one functional. Note that one of the rudimentary opercula is attached near the base of the functional one (× 15).

In attempting a provisional explanation of the compensatory regulation of the opercula it was stated in the above paper that there may be a restraining influence exerted by the fully developed operculum upon the rudimentary one which prevents the latter from attaining its full development. The removal of the functional operculum removes the restraining influence and the rudimentary continues its development. The new functional operculum in turn restricts the new bud developing in place of the old functional operculum and holds it at the rudimentary stage. The plausibility of this explanation is increased if it is found that in the ontogenetic development one operculum develops before the other, and therefore can hold the latter in check in the manner before indicated. With this object in view the investigation of the ontogenetic development was undertaken.

b. Historical Review. The first recorded observations on the development of the branchial apparatus in Serpulids which I have been able to find are those of Milne-Edwards ('45), on the development of the young Protula. He saw the larvæ attach themselves to solid objects at the bottom and sides of his dish. Here they secreted a cylindrical tube which at first was open at both ends and shorter than the length of the larva. At about the same time two lobes were differentiated at the anterior end of the larva, one on each side of the median line. At a slightly later period he thought he saw digitations of these lobes and he took them to be the beginnings of the branchiæ.

Pagenstecher ('63) gives an account of the development of the branchiæ and operculum in Spirorbis. He states that the first traces of the branchiæ are exhibited in the form of three knobs upon each of the two head lobes. The operculum is not differentiated until a later time when there is formed "der Fortsatz welcher ihn tragen soll und der von den Tentakeln durch eine Runzelung oder seichte Kerbung der Oberflache ausgezeichnet war." Judging by Pagenstecher's figure there is very little difference at the above-mentioned stage between the so-called opercular outgrowth and the other branchiæ. The figure gives two branchiæ on one side and two plus the opercular outgrowth on the other.

Fritz Müller ('64) noticed on the side of a glass vessel which he had on his study table a young Serpulid with three pairs of branchiæ, and which he took to be a member of the Protula group because of the absence of an operculum. However, a short time later he noticed that one of the branchiæ had an opercular enlargement at its end though it still retained its branchial pinnules. Still later the branchial pinnules disappeared also and he had a Serpulid with the typical genus-Serpula-type of operculum, which had developed by a modification of a branchia. In the meantime a new pair of branchiæ had been added to the oral crown making three branchiæ plus one operculum on one side and four branchiæ on the other. This is the only recorded observation of the transformation of a branchia into an operculum.

In 1866 Agassiz described the development of branchiæ and operculum in Spirorbis spirillum, Gould (not Lamarck), and made out an alternate appearance of the tentacles (branchiæ). "The first tentacle appears on the right, next comes the corresponding tentacle on the left and only later the rudiment of the odd opercular tentacle (on the right side)." The rudiment of the operculum, though at first somewhat resembling that of the tentacles, shows a difference from the start. Claparede and Mecznikow ('69), on the contrary, make out a *paired mode* of formation of the branchiæ in other species of Spirorbis. Willemoes-Suhm ('70) speaks again of an alternate mode in Spirorbis.

Giard ('76b) raised the larvæ of Salmacina Dysteri. He found two lateral head lobes each of which soon showed a threefold division. These divisions elongated to form the first three pairs of branchiæ. On each side there were two dorsal and one ventral branchia, the latter, however, dividing into two on the fifth day, so that there were then present eight branchial trunks, four on each side. The first pinnule appeared on the eighth day on the upper third of the external dorsal branchia. This is the only notice of pinnule formation I have found.

In Manayunkia, a fresh water Serpulid, Leidy ('83), describes the head lobes as showing the branchial digitations from the first trace of formation of the former.

Salensky ('83) likewise states for Pileolaria sp. that four branchiæ and the operculum appear at the same time from a median dorsal plate. The opercular "anlage" is from the beginning three to four times as large as the branchial "anlagen." In Salensky's words: "On voit d'apres cette description, que, chez Pileolaria la formation des branchies et de l'opercule s'opère en même temps, et non comme Agassiz et Pagenstecher le montrent pour Spirorbis spirillum."

Meyer ('88) describes the development of the branchiæ in Eupomatus (= Hydroides). He makes out the appearance of the two head lobes from each of which the three processes representing the first three branchiæ sprout out. The development was not carried further than this. This method of formation agrees also with that described by Roule ('85) for the larvæ of Dasychone.

From the foregoing notes it is evident that further observations on the early development of the branchiæ are necessary in order to clear up our ideas regarding the matter, and when we come to the opercular development we have practically nothing outside of the observations on the highly modified forms Spirorbis and Pileolaria except the short note of Fritz Müller upon the change of a branchia into an operculum. Regarding the formation of the rudimentary operculum there is nothing at all, and we therefore get very little aid in our study of the correlation between the two opercula at their first appearance and up to the time when they assume the final adult condition.

The following observations on H. dianthus were made at the Cold Spring Harbor Biological Laboratory on Long Island, N. Y., during July, August and September, 1902. The observations on the other species, H. uncinata and H. pectinata, were made at the Naples Zoölogical Station in the winter of 1902–03. The observations on the method of rearing the larvæ and on their general activities will be given in a separate short note.[1]

c. Observations. When the free-swimming larva is about nine days old its body is considerably elongated and shows external signs of segmentation. The apical cilium is long and two very prominent. reddish eyespots are present. (Fig. 10A.) As the movements of the animal become more and more sluggish just before its fixation to a solid object the apical cilium gradually grows smaller until it disappears entirely. At the same time three pairs of setæ are formed, the first pair being especially long and prominent. (Fig. 10B.) Fig. 10C shows a larva, 17 days old, with the tube covering about one-half of the body.[2] Here each of the two lateral head lobes already shows the division into three blunt processes, the forerunners of the branchiæ. These divisions of the head lobe appear very soon after the formation of the head lobe itself but the latter has a short separate existence before the tripartite subdivision appears. The two dorsal processes are more closely connected together than with the third and more ventral one. The relation is more clearly made out in Fig. 10D, where the mutual union of the two dorsal pairs is very evident. The two larvæ (Fig. 10C and Fig. 10D) are of the same age (17 days) and come from the same dish.

In Fig. 10E there are still the same three pairs of processes though the branchial character is more evident than before. This specimen is from the same dish as the others (16 days after fertilization). It is evident from these data that the rate of development of the different larvæ in a single dish varies within wide limits. At the stage represented in Fig. 10E the inner surfaces of the branchiæ are covered with very active cilia. In order to get at the method in which these primary processes branch later on, I have designated them arbitrarily by the Roman numerals I, II and III. I represents the most dorsal pair, II the middle pair and III the ventral pair, R or L being prefixed to represent right or left when there is any need for distinction between the two sides.

---

[1] Biological Bulletin, '05, vol. viii.

[2] The tube secreted by the animal is at first a very short cylinder which is quite transparent and covers only a small part of the larva. The ring at first is situated near the anterior end of the body just back of the eyes.

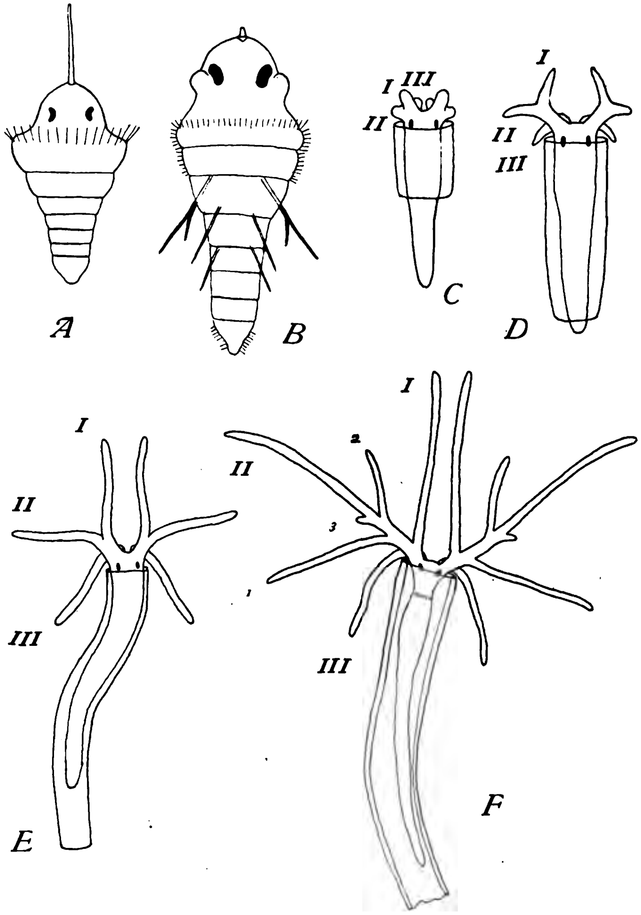


Fig. 10.

Hydroides dianthus. Larvæ. Stages of transformation from free-swimming to sedentary life. Dorsal views. *A*—Free-swimming larva. Age, 9 days. Shows long apical cilium (×208). *B*—Swimming but sluggish larva. Age, 9 days. Shows setæ and shortened apical cilium (×185). *C*—Attached larva (×90). Age, 17 days. Three pairs of head lobes. Beginning of secretion of tube. *D*—Age, 17 days (×90). *E*—Age, 16 days (×85). *F*—Age, 17 days. Second of original three pairs of branchiæ shows secondary branches (×70).

In Fig. 10F, also 17 days after fertilization, we find a stage considerably more advanced in which each of the middle branchiæ has already sent out three branches, which may be labeled according to age, II—1, II—2 and II—3. Of these it will be seen that II—1 very early takes on the character of one of the main branchial trunks, so that we thus get a stage with four pairs of branchiæ. II—2 and II—3 retain the characters of pinnules of the Branchia II. Neither I nor III has any branches at this stage.

Fig. 11A shows a later stage taken from the same dish at the same time (17 days). Here we still have no branches of I and III. Branchia II has five branches and the first branch of L-II (L-II—1) a branchlet of its own (L-II—1—1) just appearing as a very small knob. It is very evident that Branchia II is rapidly outgrowing I and III in strength.

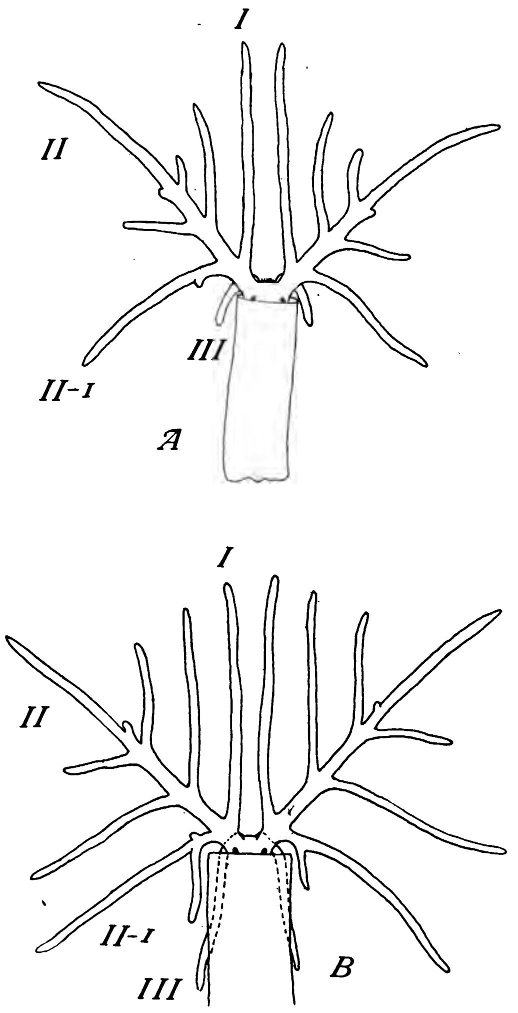


Fig. 11.
Hydroides dianthus. Dorsal views. *A*—Age, 17 days (×47). *B*—Age, 19 days (×62).

Fig. 11B (19 days old) gives a still older stage. Branchia I on each side has not increased much in size and is hardly larger than the older branches of II. L-II—1 has two branchlets (= pinnules) and R-II—1 has one branchlet. Branchia II on each side now shows the beginning of the sixth branchlet or pinnule. Branchia III also has not increased much in size.

In Fig. 12 (23 days old) Branch II—1 with its six pinnules has very evidently taken its place as a prominent part of the branchial apparatus. Branchia II has now added its eighth pinnule. Branchiæ I and III are still unbranched and have increased comparatively little in size.

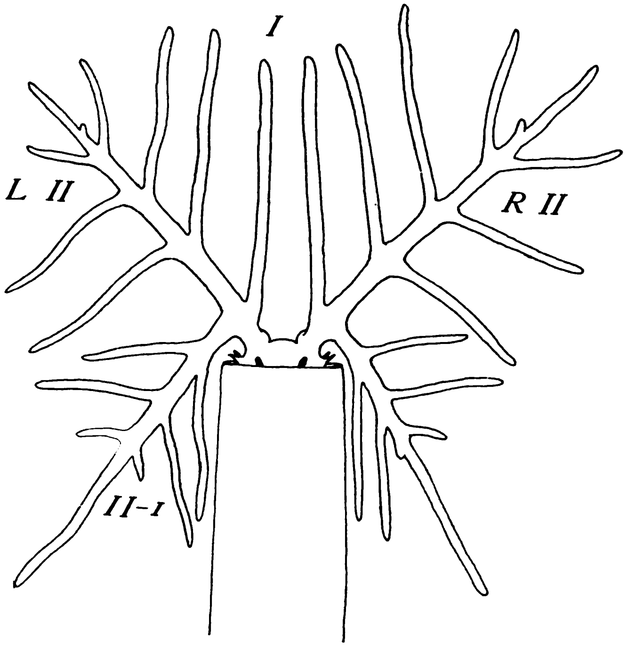


FIG. 12.

Hydroides dianthus. Age, 23 days (×75). The first secondary branch of Branch II has assumed the character of an independent main branch with branchlets of its own. The original third pair of branches is not shown.

In Fig. 13 (23 days old), in which only the right side is represented as both sides are essentially similar, Branchia I for the first time shows a trace of branching, seven little branchlets (pinnules) appearing simultaneously. Branchia II has added one new branchlet making nine in all counting Branchia II—1 as the first one. Branchia II—1 has eight pinnules. The character of Branchia III was not made out.

The operculum was seen for the first time six days later at the stage shown in Fig. 15. Fig. 14, taken five days later still (34 days old), *shows an earlier stage of the operculum. Here it appears as a rounded knob on the end of Branchia L-II.* This branchia has at this time nine branchlets counting the one (II—1) which has assumed the character of a main branch. The eight represented as pinnules are II—2 to II—9. The knob at the end of the branchia is conical in form with the base of the cone free and the apex attached to the stalk. The corresponding branchia on the right side (R-II) has likewise at this stage nine branchlets counting the independent one (II—1) or eight dependent ones (pinnules) II—2 to II—9 without this, *but there is no modification at the tip of the main stalk* as occurs on the left side.

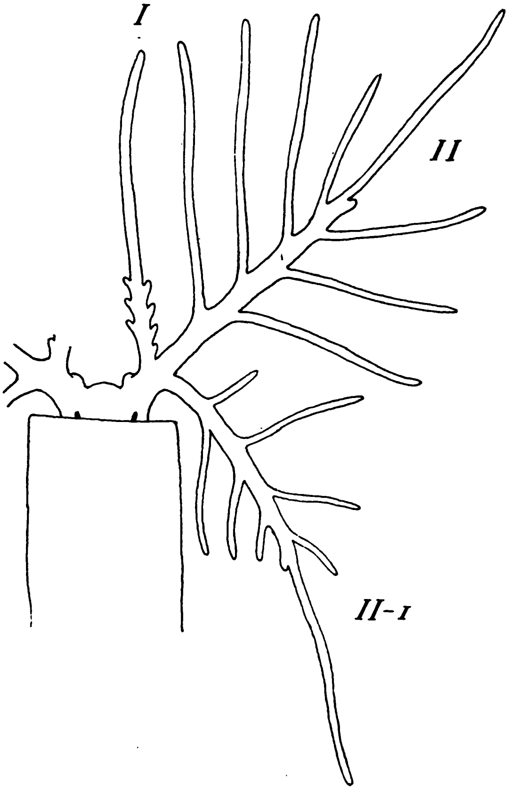


FIG. 13.

Hydroides dianthus. Age, 23 days. Dorsal view (×75). Original third pair is not shown. First pair shows beginnings of secondary branches.

In Fig. 15 the opercular character of the knob on L-II is very evident. The cup of the operculum has a notched edge with eleven serrations and resembles in its character the Serpula type and not the Hydroides type. The eight dependent (II—2 to II—9) and one independent branch are very prominently developed and evidently all of them retain their respiratory character. The corresponding branchia (R-II) on the right side has branchlets similar to those of the branchia (L-II) on the left side though it shows no opercular modifications. It is similar to the latter and different from all the other branchiæ in one essential respect. While I, II—1 and III show buds of new developing pinnules its pinnules (*i. e.*, the eight dependent ones II—2 to II—9) are all

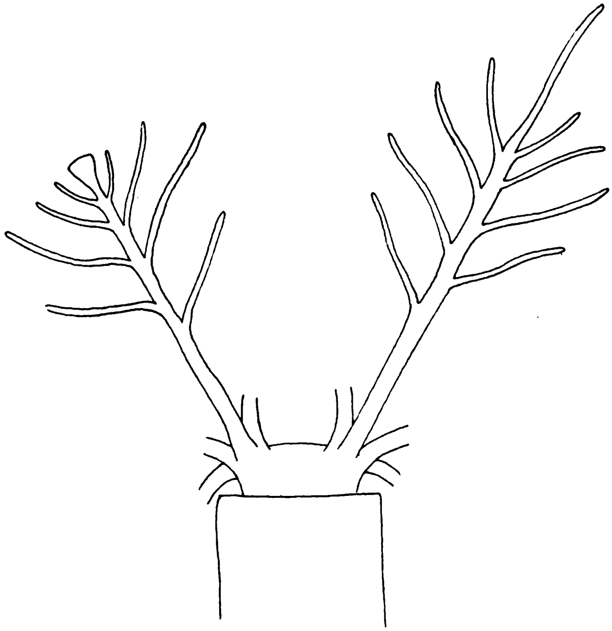


Fig. 14.

Hydroides dianthus. Age, 34 days. The next to the dorsal branchia on each side. The left one shows the beginning of the knob of the functional operculum. The right one later drops off and the rudimentary operculum regenerates from the remaining stump.

well grown, indicating, no doubt, that the organ has reached its limit of development as a branchia.

With the beginning of the opercular differentiation Branchia I enters on a new period. It increases in size and new branchlets (pinnules) sprout out in rapid succession, the first ones appearing simultaneously. Thus in Fig. 15, I already has thirteen well formed pinnules with several buds crowded into a zone at the base of the terminal filament. Branchia II—1 has likewise been increasing in size and now has 12 pinnules plus a thirteenth bud on each side Branchia III is also branched but the character of its pinnules is not given in the figure in order that complication may be avoided. This pair of branchiæ is directed away from the observer toward the ventral side of the animal. At all these stages the Branchiæ L-II and R-II both retain their flexible and branchia-like character in all respects except for the terminal enlargement in L-II.

In Fig. 16 the pinnules of L-II have disappeared, leaving a cup-like operculum with a serrated edge on the end of a long, slender, flexible but bare stalk. The corresponding branchia on the other side (R-II) has dropped off, leaving only a small round knob at the place of its former attachment. The other branchiæ, I, II—1 and III, all are keeping on with their branchial development by continually adding new pinnules to the old ones. We thus have three pairs of branchiæ at this stage with a functional operculum of the Serpula type on the left side and a rudimentary operculum on the right side. No young Serpulids were observed that showed an exception to this order of appearance of the opercula. Numerous observations were made

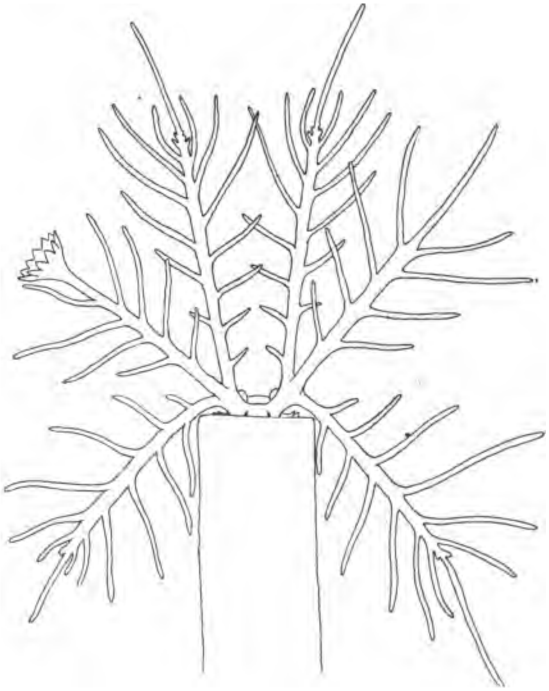


Fig. 15.

Hydroides dianthus. Age, 28 days. Shows the three most dorsal pairs of branchiæ. The ventral pair directed away from observer is not shown. The opercular knob of the left side is notched and its stalk still retains the respiratory pinnules. The next to the dorsal branchia of the right side has eight pinnules but differs from other branchiæ, except the opercular one, in the absence of new pinnule buds.

upon specimens before a count was undertaken. In this count twenty individuals were noted. All without exception, the former as well as the latter, showed the functional operculum appearing on the left side.

Fig. 17A shows the rudimentary operculum at a slightly later stage in which it has more definitely assumed its typical condition.

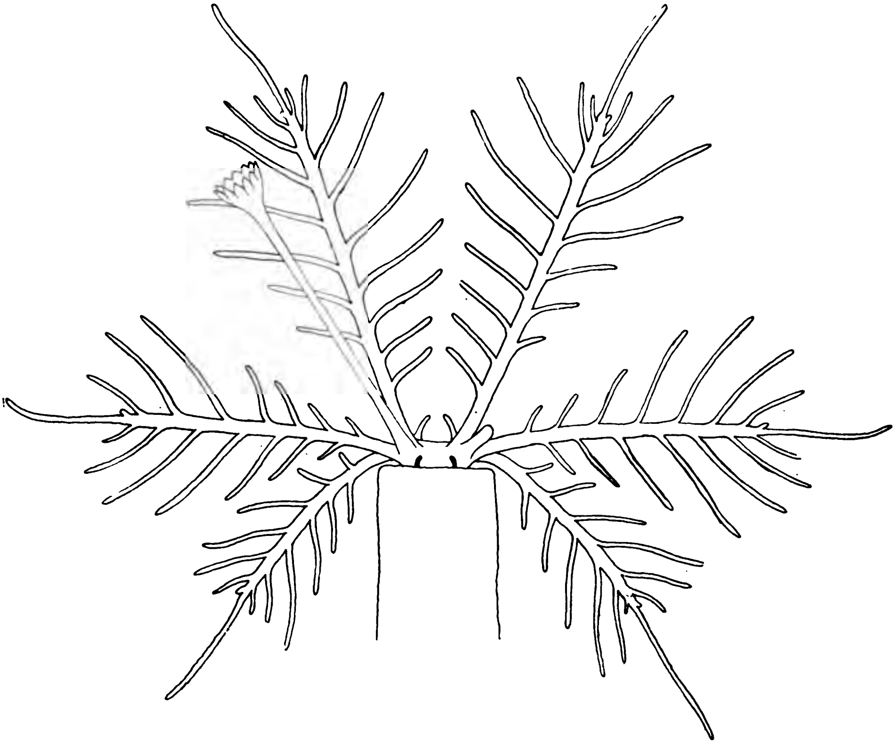


FIG. 16.

Hydroides dianthus. Age, 34 days. Dorsal view (×38). On left side is functional operculum of Serpula type with naked stalk and cup with one row of serrations. On right side is rudimentary bud developed from the base of the cast-off second branchia of that side. The three pairs of typical branchiæ also are shown.

The condition at this time resembles very closely that of the adult Serpula, as the functional operculum as shown in Fig. 16 is without doubt of the Serpula type. The further changes were not followed in H. dianthus, the species at Cold Spring Harbor, but were made out in the two Naples species, H. pectinata and H. uncinata.

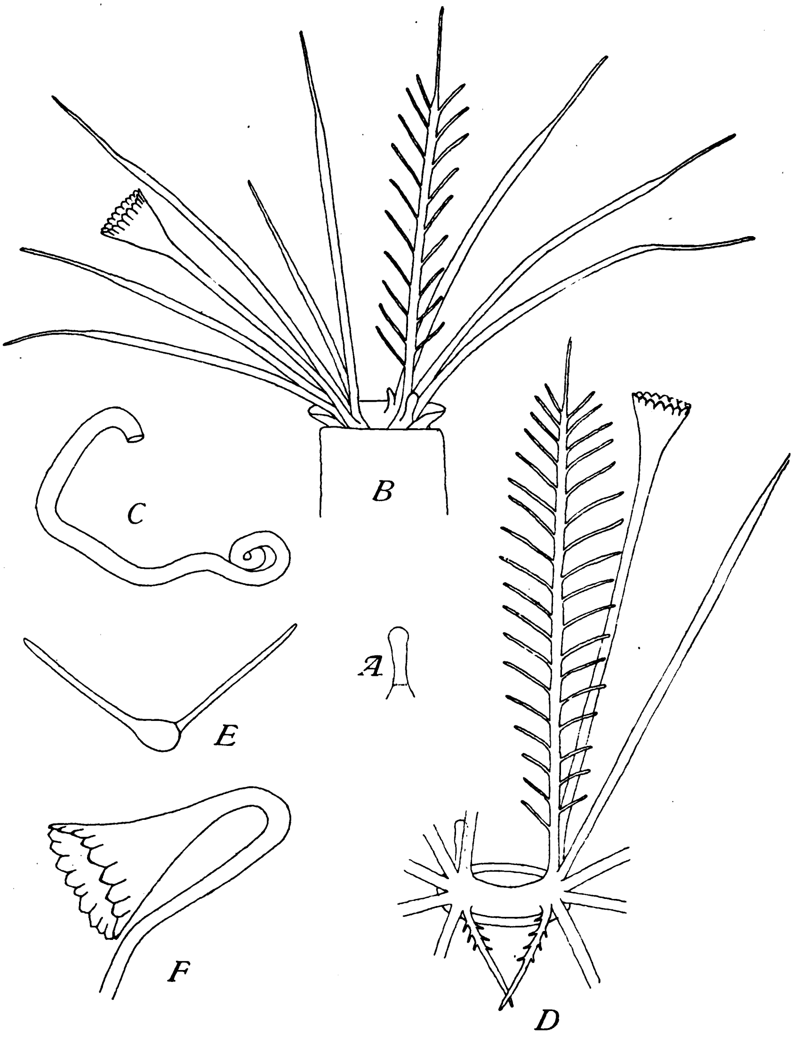


FIG. 17.

A—H. dianthus. Age, 34 days. Primary rudimentary operculum, right side (× 50). *B*—H. pectinata. Age, 45 days. Dorsal view. Functional operculum of Serpula type on left. Rudimentary operculum on right. Pinnules are shown in only one branchia, the other branchiæ being essentially similar to this one. *C*—Tube of H. pectinata. Age, 45 days (× 6). *D*—Hydroides uncinata. Age, 46 days. Ventral view. Shows Serpula type of functional operculum on left side and rudimentary operculum on right. *E*—Diagram of cross-section of a branchia of H. uncinata showing method of attachment of pinnules. *F*—H. uncinata. Age, 179 days or nearly six months. Original or Primary functional operculum as appearing after it has dropped off.

Evidently the adult condition with an operculum situated on either the right or the left side and having two rows of processes at its distal end is not fully explained by the Cold Spring Harbor observations.

Only three larvæ out of a great many sets of eggs started at Naples lived through the period of attachment. Two of these were H. uncinata and the other H. pectinata. These were first carefully observed only after they had developed up to the stage corresponding to Fig. 16 of H. dianthus.

In Fig. 17B is represented a specimen of H. pectinata with essentially the same characters as those of H. dianthus in Fig. 16. The number of branchiæ has, however, meantime increased and there are here besides the opercula five branchiæ on the left side and four plus the bud of a fifth on the right side. The functional operculum has the essential characters of the Serpula type (see above). The new branchiæ are being added on the ventral edge of each of the branchial ridges. Both opercula have moved down from the line of the branchiæ and the gap left in the line by their absence is being closed up. The character of the tube at this stage is shown in Fig. 17C. The tube was so extremely irregular in shape, largely because it was detached from the glass frequently in order to facilitate observation.

Practically the same conditions are shown at this time in H. uncinata where there are on each side five branchiæ plus the operculum. The opercula here as before have the characters of the Serpula type. (Fig. 17D.)

No further changes in the opercula were noticed for a long time. Finally, six months after the fertilization of the ova, the animals were again carefully observed, and it was noticed that the primary functional operculum (left side) had fallen off (Fig. 17F) and in its place a rudimentary one had developed, while the primary rudimentary operculum of the other (right) side had developed into a functional one. (Fig. 18A, B, C.) The two specimens of H. uncinata retained their simple Serpula-like operculum longer than did H. pectinata.

Returning to the specimen of H. pectinata as it was found after the reversal of the opercula we find all the adult characters except the full number of branchiæ. The branchiæ increase in number by additions along the ventral edge of each branchial ridge. In specimens at this stage there are beside the opercula seven branchiæ plus a very small bud on the left side and six branchiæ plus a large bud on the right. The functional operculum has a basal funnel-shaped cup with a serrated edge. From the upper flat end of this cup there projects a new secondary cup, the individual serrations of which reach nearly to the base and are strongly toothed.

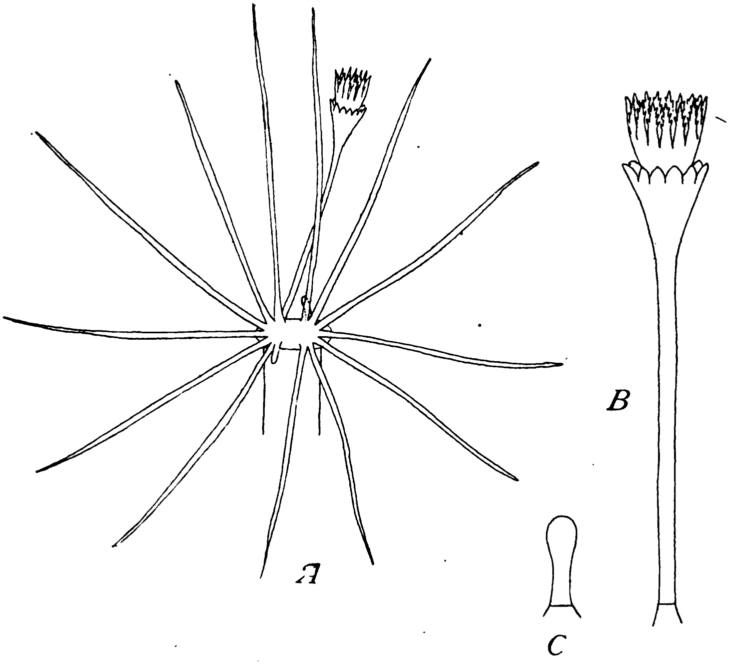


Fig. 18.

Hydroides pectinata. Age, 181 days or 6 months. *A*—Ventral (somewhat inclined) view, showing position of secondary functional and rudimentary opercula. Pinnules of branchiæ are not shown in the figure (× 16). *B*—Secondary functional operculum (adult Hydroides type with two rows of processes) (× 32). *C*—Rudimentary operculum (× 32).

d. Summary of Data and Discussion. The above results concerning the ontogenetic development of the opercula may be summarized as follows:

At the stage with four pairs of branchiæ the next to the dorsal one on each side stops its development when it has eight long slender pinnules. The two branchiæ of the third pair, counting from the dorsal side, arise originally as branches which resemble in all respects the ordinary pinnules, but instead of retaining their dependent condition increase in strength, develop secondary branches of their own and soon take their place as independent branchiæ coequal with the three primary pairs. After reaching its limit of growth as a branchia the next to the dorsalmost branchia on the *left side* starts a new differentiation at its end, developing a knob which rapidly increases in size and soon assumes the shape of an inverted cone. Along the edge of the upturned base notches appear so that the whole knob has the general character of the opercular cup of members of the genus Serpula. All this time, however, the stalk has retained its eight branchial filaments and the corresponding branchia on the other side has remained unchanged. This stage corresponds in general with the adult of Filograna or rather with Apomatus except that only one operculum is present. The branchial filaments of the stalk, however, soon disappear. Whether they drop off or are resorbed was not made out but the former supposition is the more probable one, as they were still very long a short time before they had entirely disappeared; or, in other words, no intermediate stages of resorption were seen. Almost coincidentally with the disappearance of these pinnules the next to the dorsal branchia of the right side drops off, the region of the break being near the base. From this broken stump a bud develops which in a few days has reached its limit of development for the time being. This bud, which *remains as the primary rudimentary operculum* for several months, is *a regenerated structure, a true case of physiological heteromorphosis.* Furthermore, it is restricted in its development by some forces acting from without its own substance. At this stage the opercula remain for a considerable time (several months) with no further change, although at the same time the animal is increasing in size, is building up its tube and new branchiæ are being added on the ventral edge of each of the branchial ridges. In its essential characters this stage is equivalent to that of adult members of the genus Serpula with the functional operculum on the left side.

After this long period of no opercular change the primary functional operculum drops off, the stalk breaking near its base. Immediately the primary rudimentary operculum on the right side, no longer restricted by outside forces, starts its further development and becomes a functional opercular organ. However, it does not develop into an operculum of the simple type like the primary functional one. Instead it takes on the characters of the adult Hydroides operculum with two rows of serrations. Beside the inverted cone with serrations around its upper edge there is an additional circlet of pointed and often hooked processes, which constitute the most important character of the Hydroides group as distinguished from the Serpula group. At the same time the broken stump on the left side has started to develop a knob of embryonic tissue which grows only up to the stage represented by the rudimentary operculum of the adult and is in its turn restricted in its further development by some force most likely similar to the one which in the first place restricted the original primary rudimentary operculum. There are, therefore, at this time a secondary functional operculum on the right side and a secondary rudimentary operculum on the left side. These have the essential characters of the opercula of adult specimens of Hydroides. However, one point of difference is evident in specimens taken at random from the sea. It is found that approximately the same number have the operculum on the left side as on the right though there is uniformly a slight advantage in favor of the right-handed ones (57 per cent right-handed to 43 per cent left-handed in H. dianthus), while all the larvæ appeared first as left-handed ones and later by reversal changed to right-handed ones. How does this change occur? Either we must suppose that the similarity in character of all the larvæ was accidental or that the reversal takes place in nature during the life of the individual more than the one time described for the young animal. The first supposition seems improbable because the larvæ came from a great many different individuals, and moreover the order of appearance was found to be the same in the two Naples species (H. uncinata and H. pectinata). We are, therefore, forced to assume a further reversal as taking place in nature. This reversal may be a purely physiological one, induced by the normal activities of the animal, like the first reversal already described, or may be induced by some injury to the functional operculum of the character which was found to cause such a reversal in my experiments. (See below, p. 55 *ffl.*) However, unfortunately, there is no experimental evidence to show that physiological reversal takes place in nature after the first time already described. As is mentioned elsewhere (p. 65) in this paper the experiments undertaken to determine whether worms kept in dishes in the laboratory exhibited physiological reversal were negative, although the time was in all cases too short to constitute a good test. There was no change in any case unless the functional operculum was injured. However, the very near equality between right and left-handed individuals seems to preclude the possibility of all reversal being due to injury of the functional operculum. And we have beside the analogous case of physiological reversal in the young animals as has been emphasized before.

2. Regeneratory Development.

a. Introduction. The *nature* of the opercular modifications among the Serpulids has now been outlined in the discussion of the comparative anatomy and their *origin* within the individual life history has been traced in the genus Hydroides. There now remains an attempt at an *experimental analysis* of the factors involved in the development and maintenance of the adult characters. Partly because of the imperfection of the method and partly because it is not desirable to dissect the data too minutely while presenting them, the latter will be given in the descriptive portion of this section as parts of individual experiments without perfect regard to logical development of the analysis of the factors involved. The latter will be attempted more fully in the general discussions to follow the descriptive portions.

In a former paper a preliminary report of some of my experimental results on compensatory regulation in the regeneration of Hydroides dianthus was given. Since that time a more detailed series of experiments has been undertaken along the same lines and the work has been extended to other species of the family. Two distinct problems have come up. In the first place is the study of the factors involved in the compensatory regulation of the opercula which, as stated above, is the main object of the present paper. In the second place a comparison of the regeneratory development of the opercula with the ontogenetic and with the probable phylogenetic development brings up an extremely interesting discussion with important bearings on the recapitulation theory.

The great majority of the experiments were performed on H. dianthus and this form will be discussed first. Then will follow the other members of Group V, namely, H. uncinata,

H. pectinata and Serpula vermicularis, then a member of Group IV, Apomatus ampullifera, and finally the members of Group VI, Ditrupa subulata, Spirorbis Pagenstecheri, Pomatoceros triquetroides and Vermilia multivaricosa. The adult condition of the opercula which has already been described in detail in the anatomical portion of the paper will be again briefly noted, as it must serve as the basis of our experiments. The experiments will then be described in turn, and finally the results will be discussed.

b. Unoperated Condition of the Opercula in Hydroides Dianthus. The character of the adult opercula has already been given on p. 21 *ffl.* and is also shown in Fig. 5E, F. A repetition of these data is therefore unnecessary.

In all the experiments about to be described the animal was first removed from its tube and placed in a dish of sea-water, the desired operation was performed under a dissecting microscope and the animal was kept in its individual dish either with running or standing water, in the latter case the water being changed once or twice a day as required. The running water was not found as favorable as the standing because of the collection of a fine deposit on the animals notwithstanding the greatest care exercised. The dishes with standing water were found very favorable if provided with a glass cover to keep out the dust. The observations were in most cases made on the living animals.

c. Operations on Functional Operculum. The results may best be arranged around a description of the effect of a cross cut through the stalk three-fourths of the distance from the base to the beginning of the terminal expansion. The stump of the functional stalk remains attached to the animal for two or three days as a rule or even longer in some cases. It then breaks off from the body, separating by a clean division at the basal suture or "breaking joint" described above (pp. 21–23). On the distal end of the small stump still remaining attached to the animal a small bud now appears and gradually increases in size until it reaches the dimensions and character of *the former rudimentary operculum of the opposite side.* At this point it stops and proceeds no further. In order to understand the result it is necessary to look away from the immediate vicinity of the operated organ and to note the change going on in a corresponding position on the other side of the animal. Even before the attached stump of the operated functional operculum has fallen off the rudimentary operculum has started to enlarge and processes appear at its distal end. Gradually the structure increases in size and assumes

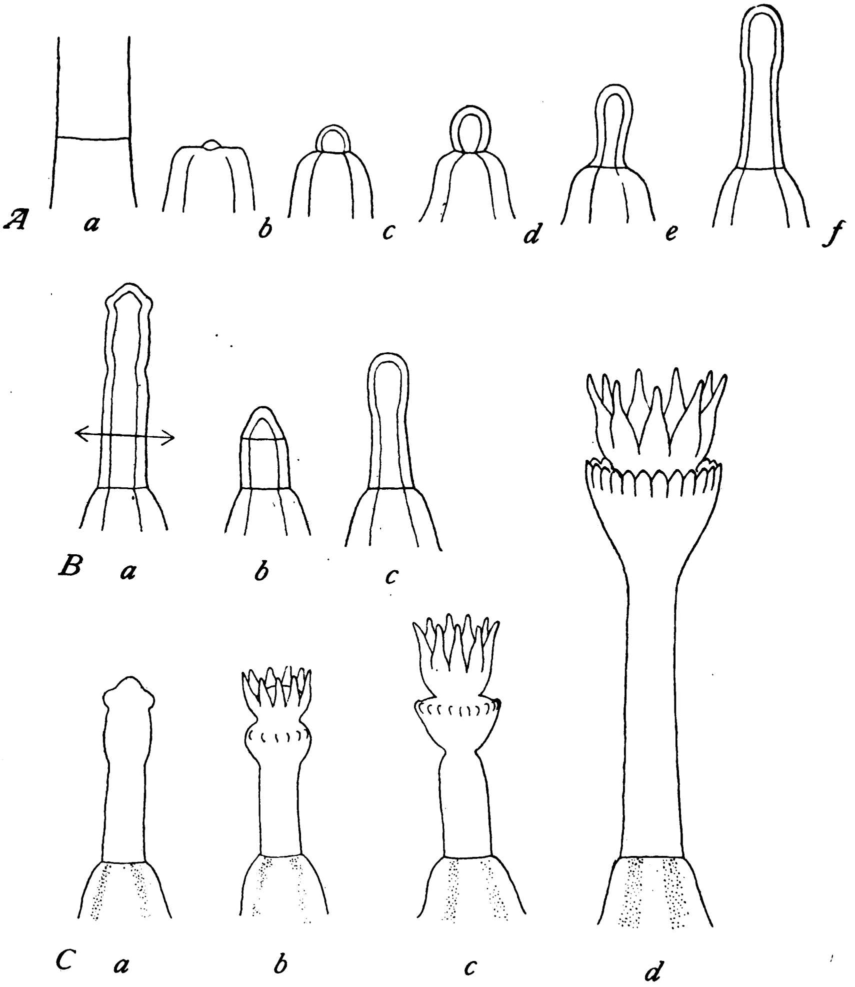


Fig. 19.

Hydroides dianthus. *A, a–f*—Stages in the development of a rudimentary operculum from the stump of an old functional one (× 54). *B, a–e*—Stages in the regeneration of a rudimentary operculum after a cut at the region indicated by the double-headed arrow (× 54). *C, a–d*—Stages in the transformation of the old rudimentary operculum into the new functional after removal of the old functional operculum. The final condition of the functional operculum is shown in Fig. 5F.

more and more the *character of the former functional operculum.* After 15 to 20 days the development is complete and we have a complete reversal of the opercula. The former functional operculum is now the rudimentary and the former rudimentary has become the functional. The resulting arrangement is the exact reciprocal of the former one.

The case just briefly outlined will now be taken up in more detail. Before the stalk of the functional operculum is cut it is almost impossible to pull it off from the animal. The basal suture resists breaking as well as the solid material of the stalk. The hardest kind of a pull that can be given with a pair of forceps is not sufficient to dislodge the organ. Inside of a few days after the operation, however, the opercular stalk as we have seen comes off of its own accord, so that great changes must be assumed to have taken place in the region. The time at which the stalk drops off varies greatly. In one case it had not come off 5 days after the operation and in another after 6 days it was still attached. Very soon after the stalk has dropped off a bud appears at the top of the stump and this steadily increases in size until the typical form of a rudimentary operculum is reached. (Fig. 19 A, a–f.) It seems very probable that the breaking off of the remanent of the stalk is induced by histological changes in the suture region which are attendant upon the beginning of the development of the new bud. Evidence in favor of this view will be given later in another place.

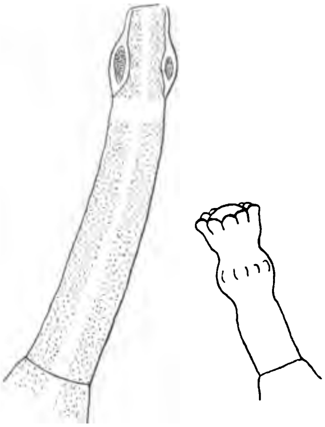


Fig. 20.

Opercula of Hydroides dianthus, five days after transverse section of stalk (×26). Exceptional case. Differentiation at the distal end of a functional stalk which had been cut two-thirds of the distance from the base to the terminal cup. The old rudimentary is also shown.

Except in one case no differentiation of tissues took place at the cut end of the functional stalk. In this case there was an expansion on each of the two sides of the stalk near its tip. The stalk did not drop off for 5 days after the operation. The changes are represented in Fig. 20.

The changes taking place on the other side of the body are very evident 3 or 4 days after the operation, often before the remanent of the functional stalk has dropped off. This fact that the rudimentary operculum begins to develop even before the functional has dropped off seems to argue against the mere retarding influence of the organ as a mechanical weight, though of course the weight is considerably diminished by the removal of the part of the operculum distal to the cut. The stages through which the rudimentary operculum passes in changing into a functional one are given in the accompanying figures. (Fig. 19c, a–d.) The essential points to be emphasized in this development are: first, *the fact that throughout there is no sign of the appearance of special branchial characters*, and, second, *that the secondary processes of the operculum appear before the primary ones*. The regeneratory development, therefore, differs widely from the ontogenetic or probable phylogenetic one as regards these points.

A series of operations was performed on the functional operculum to determine the amount of injury necessary to bring about reversal. The cases overlap slightly but it is found that in general the cutting off of the distal circlet of processes does not induce reversal while cuts through the main enlarged portion of the operculum bring about a reversal of the opercula. In one case the stalk of the injured operculum remained attached though the rudimentary operculum in the meantime had reached a stage equal to three-fourths of the normal functional development. It may be concluded that *a removal of the secondary circlet of processes of the functional operculum does not as a rule cause reversal, while a similar injury below this point to the main portion of the cup or to the stalk of the operculum always brings about such a result.*

d. Operations on Rudimentary Operculum. When the rudimentary operculum alone is removed there is no effect upon the functional operculum and a new rudimentary develops in place of the old one which had been cut off. The cut end rounds off, a bud-like mass of new tissue appears there, and the whole, both old and new tissue together, gradually assumes the shape of the old rudimentary operculum. The greater part of the change from the beginning is, however, accomplished by the growth of new tissue and only very little by the change in form of the old. No further development takes place. The result is the same no matter what the level of the cut may be. One of the levels at which cuts were made in my experiments is shown in the accompanying figure. In none of them did the functional operculum change its character or did any structure other than a rudimentary operculum develop in place of the old rudimentary. (Fig. 19B, a–c.)

e. Operations on Both Opercula. When both opercula were cut off it was found that while in some cases there was a reversal, in others two functional opercula were developed, one on each side, while in still others characteristics differing from either of these two combinations were formed. An attempt was made to find the cause of the difference in results and with this object in view cuts were made at different levels. The difference of level in the cuts in the rudimentary operculum could not be easily controlled, but since in the former cases where only the rudimentary operculum was removed there was no specific influence either on the opposite functional operculum or on the new regenerating one, it is supposed that these differences of level have here also no influence upon the character of the result. In every case care was taken to bring the cut well down toward the base of the rudimentary operculum. The different levels on the functional operculum are indicated in Fig. 21. A summary of the results is made in Table VIII.

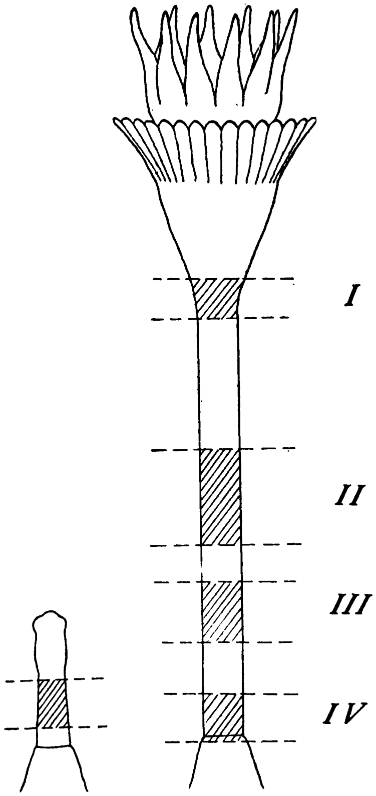


Fig. 21.

Hydroides dianthus (× 30). Operations made on both opercula simultaneously. I, II, III, IV represent the regions of functional operculum in which the cuts of the four groups of experiments were made. The shaded portion of the rudimentary operculum between the two dotted lines includes all the cuts on this side.

If we neglect the differences in the levels of the cuts in the rudimentary operculum and divide those of the functional one into four groups we get a very interesting correlation between the regions and the corresponding results of the operation. In this way we get four fairly well marked groups. Group I consists of accurately located cases near the distal end of the stalk where it expands into the cup. Group II has the earlier cases located from description alone, in most cases stating that the "functional stalk was cut near its middle." Group III has accurately determined cases located about one-fifth of the length of the opercular stalk from the basal suture. Finally, Group IV contains the cuts made just distal to the basal suture. (Fig. 21.)

TABLE VIII. *Hydroides dianthus. Both Opercula Removed.*

	$F_1 = F_2$ $R_1 = R_2$	$F_1 = R_2$ $R_1 = F_2$	$F_1 = F_2$ $R_1 = F_2$	$F_1 = S$ $R_1 = F_2$	$F_1 = r'$ $R_1 = r''$
Group I..........	0	3	0	0	0
Group II..........	1	1	3	1	1
Group III..........	0	1	4	0	0
Group IV..........	0	5	0	0	0

F_1= Original functional operculum.
R_1= Original rudimentary operculum.
F_2= Resultant functional operculum.
R_2= Resultant rudimentary operculum.
S = Functional stalk remains attached.
r', r''= Small undifferentiated buds of new tissue.

In Group I the three valid cases (see Table VIII) all showed a reversal of the opercula, the old functional becoming the new rudimentary and the old rudimentary becoming the new functional.

In Group II where the region of the cut was not so accurately located the results are scattering. Seven of the cases give results valid for our purpose. Of these three developed two functional opercula, one showed a reversal similar to that of Group I, one showed the development of a new functional in place of the old and a new rudimentary in place of the old rudimentary, in one the functional stalk did not become detached and the old rudimentary developed into a functional, and in still another case there were rudimentary buds on both sides, neither of which reached a stage beyond a rounded knob and were, therefore, not developed even up to the rudimentary stage proper.

In Group III, consisting of accurately determined levels, four of the five cases showing valid results developed two functional opercula, the fifth one showed a reversal of the opercula.

In Group IV, also consisting of accurately determined levels, five cases showed a clear result and all of them had a reversal of the opercula.

The result is a peculiar one in that *the most distal and the most proximal groups agree in giving rise to a reversal of the opercula, while the intermediate two groups give rise in a majority of the cases to two functional opercula.*

An attempt at an explanation is hazardous and can be little more than a guess. Such a provisional attempt may, however, be made, for by so doing some light may be thrown on the regulation of the "normal" condition in the animal. An examination of the whole number of cases where both opercula are cut off shows that in all but two the rudimentary operculum after regeneration did not stop at the rudimentary stage but kept on developing until it reached the functional stage. The difference in the results is then due to differences in the regeneratory development of the old functional operculum. What factor or factors hold it in the rudimentary stage in some cases, while in others it is allowed to develop into a full-sized functional organ? Two factors are to be considered: first, the influence of the position of the cut upon the initial stages of change in the embryonic tissue in the neighborhood of the basal suture and, second, the possibility of a retarding influence emanating from the new functional operculum which is rapidly developing on the opposite side from the stump of the old rudimentary.

First Factor. It has been shown above (p. 58), in the series of experiments with an uninjured rudimentary operculum, that injury to the extreme distal portion of the functional operculum does not lead to the dropping off of the injured organ or to the development of the opposite rudimentary operculum into a functional. Further, from the same series of experiments it is seen that the development of the opposite rudimentary operculum is more easily induced by a terminal injury to the old functional than is the dropping off of the old functional stalk. The latter point is well illustrated by several cases in which the injured stalk remained attached to the animal while the opposite rudimentary had already developed into a full sized new functional. From these data we may assume that the distal cuts do not affect the embryonic tissue at the basal suture as quickly as do the more proximal cuts and for this reason the tissue will have had only a small start when the opposite rudimentary already has a very considerable one. The latter may then restrict the further development of the bud after the old stalk has fallen off. In Group IV, on the other hand, the cut is so near the embryonic tissue of the suture itself that it may directly injure the cells which are to give rise to the mechanism of a cleaving plane or else leave such short leverage in the distal portion of the stalk as to compel the growing tissue to do all the work in pushing off the useless portion and thus to retard its growth. It is possible also that in some of the cases in Group IV the short portion distal to the suture is not cast off, but that the tissues are re-formed and thus give rise to the growing bud. However, no observations were made on this last point and it is merely put down as a possibility in view of some of the later experiments on *Pomatoceros* (p. 70).

In the above paragraph an attempt has been made to show how the embryonic tissues at the basal suture in Group I and in Group IV may develop up to the stage of a rudimentary operculum less rapidly than those of Groups II and III.

Second Factor. Admitting this greater development of the bud of the old functional side in Groups II and III than in Groups I and IV, and further assuming the uniform development of the bud of the old rudimentary side in all four groups, we are led to the consideration that if one of the opercula when well developed compels the other to stay in a rudimentary stage such a cause may act in Groups I and IV and not in Groups II and III. *Therefore, in the former cases, the result of the simultaneous operation on both opercula is a reversal of the original condition, while in the latter it is the production of two "functional" opercula.*

f. Body Cut in Two. Regeneration of Opercula at the Anterior End. When the body is cut in two in the thoracic region two opercula and groups of branchiæ are regenerated on the two sides of the median line but the opercula instead of being differentiated into a large one and a small one are both of the large functional type. We must assume in this case that since both had an equal start in development the retarding influence of the one upon the other which occurs in other cases did not occur here.

The newly developed opercula were in some cases exact duplicates, the one of the other, but in others one operculum was considerably larger than the other, though both showed the true "functional" characters. The extremes of the different cases are given in the accompanying figure. (Fig. 22.)

The resultant opercula usually differed from the normal functional one in being shorter and stubbier than the latter. It is to be noted that two fully developed opercula of the kind indicated can be of little value in closing up the opening of the tube as each one stands in the way of the other. The animals in the experiments were, however, not kept in their tubes so that the actions under such circumstances were not observed. It seems that the anterior missing segments were not regenerated in any case. Whether such regeneration would be possible under favorable conditions cannot be said. In my specimens bacteria and infusoria developed on the tender regenerating tissues and the growth was retarded and finally stopped.

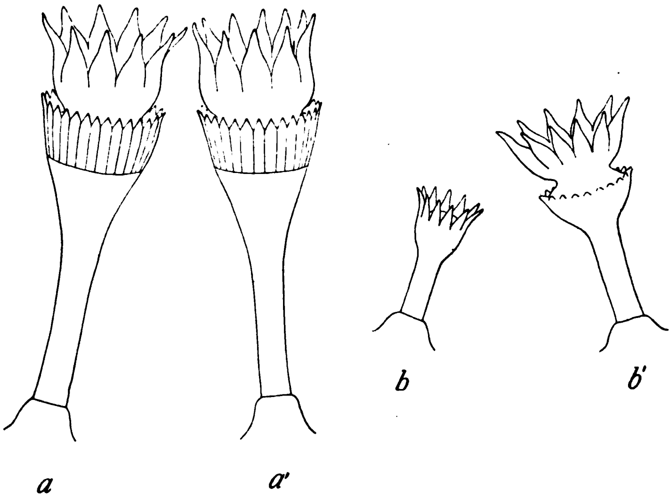


FIG. 22.

Hydroides dianthus. Opercula as regenerated at the anterior end of the posterior piece after transverse section in the thoracic region (× 17). Left, case with equal opercula. Right, case with unequal opercula.

The main point as regards the opercula made out in the group of experiments where the body was cut in two in the thoracic region is this: *When the opercular buds have an equal start in development both develop into functional opercula.*

The relation of the developing opercula and branchiæ of each side to the nerve cord of that side is very interesting. This is most noticeable in the regeneration of these organs from a cut near the posterior end of the thorax where the nerve cords are widely separated. In the Serpulidæ it will be remembered the nerve cords do not come together ventrally as in most Annelids but remain widely separated, forming two latero-ventral trunks. The principal blood vessels, however, do not have this arrangement. The branchial circlets, each with its operculum, regenerating from a cut near the posterior end of the thorax, are always widely separated and seem to be located in intimate relation with the nerve trunks of the corresponding sides. This fact agrees very well with the data as made out by Morgan ('02) for the regeneration of the head of the earthworm which showed that the regenerating head always develops in connection with the anterior cut end of the nerve cord. A similar relation has been made out for other forms. A further discussion of this and other cases of nervous control in regeneration is reserved for a future time.

The results of a *transverse cut in the abdominal region* were in every case negative as far as the posterior piece is concerned. Its anterior cut surface in every case healed over and no regeneration took place. The piece lived for a considerable time but did not show any signs of regenerating tissue.

In this connection two other groups of experiments may be described.

The first concerns the regeneration and regulation following the *longitudinal dorso-ventral division of the body* into equal right and left parts.

In this group a dorso-ventral longitudinal cut divided the body into approximately equal right and left halves. Fourteen specimens were operated on in this way and of these several showed traces of the regeneration of knob-like elevations near the anterior end of the cut surface. Two of these showed especially clear structures which correspond very well with young regenerating branchial circlets from the anterior end of a posterior piece after transverse section of the thorax. It is probable that the new structures may be located at a cut end of a nerve cord. In one of the cases the new circlet in question was a considerable distance behind the old branchial circlet. In no case did the animal live long enough to allow of a full development of the new organs.

The effect of the cut upon the old organs is as follows: The rudimentary operculum in several cases showed a slight development though only one advanced to the stage with both rows of opercular processes present. Usually the rudimentary operculum remained unchanged or a slight development of the secondary processes took place. No changes were observed under similar circumstances in the functional operculum.

The second group of experiments concerns the regeneration from a posterior cut surface of a half (right or left) Serpulid. Two of the cases of regeneration at the posterior cut surface after longitudinal dorso-ventral section of the whole organism showed very clearly the character of the new tail bud regenerating there. After transverse section of the whole body in the abdominal region the new tail bud is always very evidently double. In the present experiments, however, where only half of the animal was used the regenerating bud always showed a single tail knob. As the two components of the ventral nerve-cord in Serpulids and Sabellids are widely separated this singleness of structure may be correlated with the presence of only one of these nerve cords at the posterior end when the animal is cut in two longitudinally before the cross cut is made.

g. Progressive Changes in Opercula. Progressive changes in the opercula of adult specimens were not observed. Several groups of specimens were kept under observation for varying periods of time but in no case was evidence of such a change noted. It must, however, be stated that the periods were all relatively short, not over a month at most. The indirect arguments in favor of the occurrence of such changes as furnished by specimens in nature with intermediate stages of reversal, etc., are given elsewhere. (p. 33.)

h. Experiments on Group V. (See p. 26 for definition.) A series of experiments on H. uncinata was undertaken with the object of determining the relative capacity for regeneration at different levels in the body. The most posterior region showing regeneration of branchial and opercular structures was an anterior cut surface located between the next to the last (sixth) and the last (seventh) thoracic segments. Several posterior pieces back of this point lived for a sufficient length of time to allow of regeneration if it were to occur at all, but all these healed up at the cut surface and showed no regeneration of head structures. We

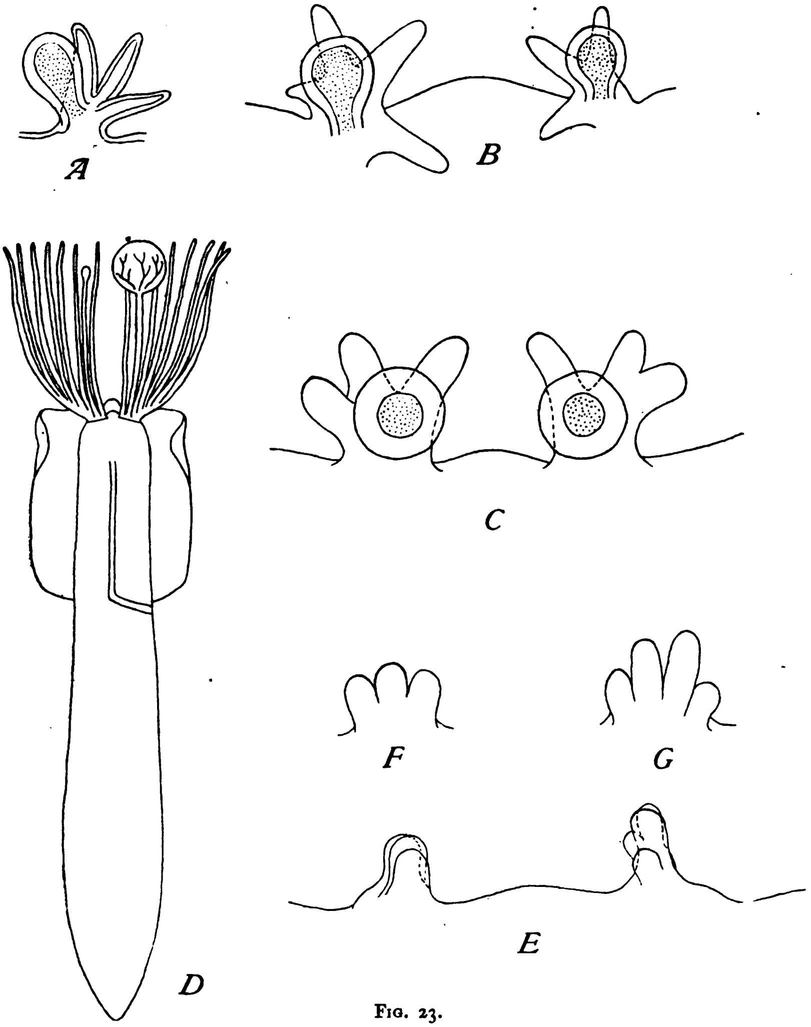


Fig. 23.

A, B—Hydroides uncinata. Regenerating branchiæ and opercula at anterior cut surface after transverse section between fourth and fifth thoracic segments (×93). *A*—13 days after operation. *B*—14 days after operation. *C*—H. uncinata. Regenerating branchiæ and opercula at anterior cut surface after transverse section between first and second thoracic segments, 18 days after operation (×60). *D*—Dorsal view of Apomatus ampullifera showing functional and rudimentary opercula (×5). Pinnules not represented. (See also Fig. 6 D, E, F).

E, F, G—Apomatus ampullifera. Regenerating branchiæ and opercula at anterior cut surface after transverse section between the third and fourth thoracic segments, 23 days after operation (×60). *E*—Dorsal view of both branchial groups. *F*—Left group as viewed from right side. *G*—Right group as viewed from left side.

must, therefore, for lack of positive evidence to the contrary decide that in H. uncinata the power to regenerate head structures is found only in the thoracic region and that anterior surfaces of the posterior pieces after transverse section through the abdomen do not possess this power.

The manner in which the regeneration takes place is extremely interesting when compared with the development of the same structures in ontogeny. It was found that in each of the cases at the earliest stages three branchial buds and one opercular bud were present on each side. (Fig. 23A, B, C.) *This corresponds with the number present in the ontogenetic development of Hydroides at the first appearance of the opercula.* A similar relation holds for the regeneration of the branchial circlets of Apomatus after a transverse cut in the thoracic region.

A series of experiments was performed on H. pectinata to determine whether the cutting of the animal in two by a transverse cut through the second and third segments of the thorax would cause any changes in the opercula remaining at the anterior end. In this series the opercula were not disturbed. The result was not completely satisfactory because most of the specimens died at an early stage but it was found that the cut did not cause the opercula to change. A severe bodily injury, therefore, need not cause a reversal.

Another series was undertaken in the hope of finding the influence of sectioning of the thorax upon the differentiation of the opercula after the functional stalk had been cut at its middle. It was found that the separation of the region of the body back of the fourth thoracic segment from the rest *does not retard* the changes of reversal in the opercula which usually take place after a section of the functional stalk at its middle.

A single specimen of Serpula vermicularis was operated on. Both opercula were cut off, the functional one at its middle. The result was a reversal of the former condition. The animal was kept in its dish unobserved for about three months and was then found to have reversed back again to its original condition.

i. Experiments on Group IV. Apomatus ampullifera. Two characteristics of the branchiæ and opercula of Apomatus need to be taken into account before going on with a description of the experiments. (See also description, p. 26.)

In the first place there are two opercula, one a large spherical body and the other a very small terminal enlargement, each at the end of the branchial stalk occupying the next to the dorsal position in its branchial circlet. This stalk is in each case a typical branchia except for the opercular enlargement and apparently carries on its full respiratory as well as its opercular function. (Fig. 23D.)

In the second place each branchial semicirclet taken as a whole breaks off very readily along a definite line at its base so that all the branchiæ including the opercular one come off together. Thus a very slight irritation is sufficient to cause the animal to throw off the whole branchial apparatus, including the opercula. The fission plane is in a very definite region at the base of the branchial circlet and after coming off the whole branchial crown holds together in one piece because of the union of the branchiæ near their bases. The right and left branchial circlets act independently in the matter since it often happens that only one is cast off. Usually, however, both are thrown off. Such an operation as the removal of the animal from its tube usually brings about this autotomy of the branchiæ. Out of 42 specimens removed from their tubes on November 6, 1902, thirty lost both branchial circlets, 6 lost one of the circlets and only 6 retained the whole branchial crown. For this reason it was not possible to repeat the ordinary operculum reversal experiments on Apomatus as after such an operation the branchial circlet was cast off.

After the casting off of the branchial crowns in these cases a regeneration of two functional opercula usually followed, though one was often larger than the other. Probably correlated with the differentiation of a "breaking joint" at the base of the circlet is the fact that the regeneration of the branchial crown does not show only three branchiæ plus the operculum at the first differentiation as in Hydroides but at once brings out several branchiæ on each side. One of these may show the opercular differentiation from the start, while in other cases it develops first as a branchia and only later shows the opercular character.

The regeneration at the anterior end of a posterior piece after transverse section through the thoracic region showed a less highly differentiated character of the new organ at the start than when the regeneration took place from the "breaking joint." A great number of operations were made, but the animal proved very sensitive to the injury and nearly all the individuals died very early. However, the beginning of the process of regeneration was observed in a few cases. In one of these which had been cut through the third thoracic segment there was, 23 days after the operation, a distinct indication of the young branchial circlets at the anterior end of the posterior piece in the form of *three branchial knobs on the left side and four on the right.* (Fig. 23E, F, G.) Of the latter four the ventral one was very small and the next to the dorsal one evidently larger than the others and showing thus early its opercular character. The regenerating tissue appears first as an undifferentiated mound. When differentiation does occur it takes on the form of three or four knobs in each mound, corresponding evidently with those of Hydroides dianthus after a similar section and reminding one strongly of the first branchial differentiation in the young of the latter species where, as we have seen, each branchial circlet appears first as three processes, one of which divides at its base, forming four in all. The early differentiation of the opercular knob after a thoracic cut in Apomatus as in Hydroides, however, brings in a point of difference as compared with the ontogenetic development.

j. Experiments on Group VI. In four specimens of Ditrupa subulata the functional stalk was cut in two just below the terminal cup. The embryonic tissues at the base of the stalk increase in bulk and bulge out, showing an oblique suture. The stalk drops off a few days after the operation and a new operculum develops from its stump. Evidently the increase in the embryonic tissues serves as a mechanical stimulus for the dropping off of the old stalk. See Fig. 24A, B.

All the specimens of Spirorbis Pagenstecheri in which the operculum was removed died. There is, however, evidence that regeneration of the operculum takes place. A considerable number of the specimens just removed from their tubes showed stages of growth of the operculum from a small bud to a large full-sized operculum. Whether the process is a direct physiological one or is due to injury cannot of course be definitely stated. A periodic replacement may be connected with a possible periodic injury during the breaking out of the embryos from the brood pouch, though evidence is also lacking as to the length of life of the animals.

Several experiments on the regeneration of the opercula in

Pomatoceros triquetroides were started. It will be remembered that the operculum has a distinct basal suture. In about half of the specimens which were removed from their tubes it was found that the operculum had been thrown off at this basal suture, the distal portion of the operculum remaining in the tube. The plane of the fracture is not a straight one but the middle is pointed forward so as to give in a dorsal view the form of an inverted Λ. Figs. 8B, C; 24C, D, E. Three series of operations were carried out. In the *first* the operculum was cut in two distal to the basal suture. Here it seems that the part of the operculum above the suture did not drop off but the regeneration took place by a growth from the cut surface. The first sign of this regeneration was a swelling of the terminal region from which three knobs developed, which evidently became the terminal processes of the new operculum. The evidence for this change and for the later changes in general is not complete as the later stages were not followed out.

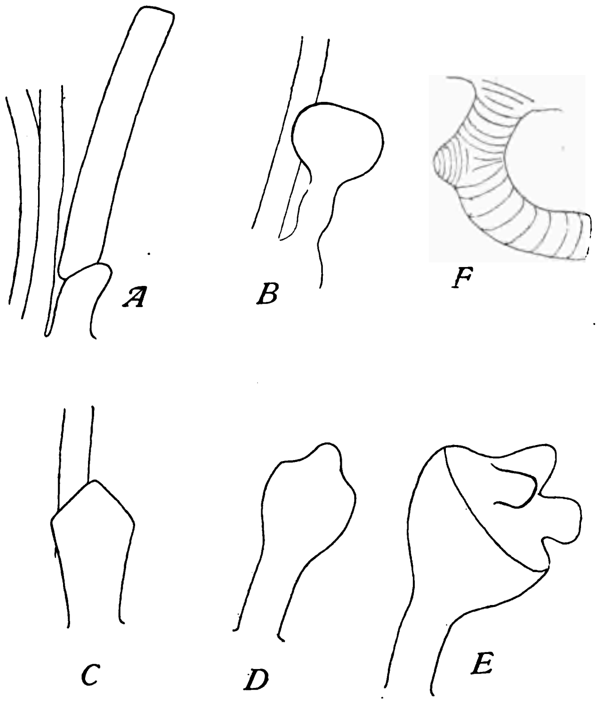


Fig. 24.

A—Ditrupa subulata. Stalk, two days after operation, showing projection of new tissue at one side of basal portion below breaking joint (×20). *B*—Regenerating operculum of D. subulata, 9 days after operation (× 20).

C, D, E—Pomatoceros triquetroides. Stages in regeneration of new operculum from breaking joint level (× 10). *C*—Operculum just pulled off. *D*—3 days after operation. *E*—8 days after operation.

F—Vermilia multivaricosa. Stalk of operculum two days after removal of cup (× 16). Note projecting knob of new tissue at side of stalk.

The interesting general point is that regeneration takes place from the cut surface without a breaking off at the basal suture.

When the operculum was pulled off at the time of removal of the animal from its tube the break always took place at the Λ-shaped suture and the regeneration then naturally followed from this level.

In a third set of experiments the animal was cut in two in the thoracic region. All such specimens, however, died before the appearance of regeneratory changes.

The operculum was removed in five specimens of Vermilia multivaricosa. In no case was there any regeneration of the organ. In one individual the cut was made through the narrow portion of the stalk just below the terminal cup. In this case (Fig. 24F), two days after the operation, there was a protruding knob on the median side of the stalk which may represent the beginning of an opercular regeneration, such as that shown in the case of Ditrupa. (Fig. 24.) In the other four specimens the cut was through the cup portion of the operculum. In all of these there was no regeneration, though three of them lived more than eleven days after the operation.

k. Discussion of the Data. It has been seen that the character of the regeneratory process varies according to the location of the cut. When the regeneration takes place from the breaking joint of the operculum (Hydroides, etc.) or of the branchial circlet (Apomatus) the regeneration is highly specialized and the stages do not follow the ontogenetic ones very closely. When, however, the regeneration is from a thoracic cut, where the branchial and opercular tissues are not as highly specialized with respect to the mechanism of regeneration, the organs pass through a stage which may very well be compared with a corresponding stage in the ontogeny of Hydroides. However, even here the regeneratory development does not follow the other closely because the operculum is very evidently differentiated as such from the start in regeneration though not in ontogeny.

Our general conclusion may, therefore, be that when there is no definite mechanism for the autotomy of a region of the body the regenerating tissue may in its various stages resemble ontogenetic stages quite closely, but where a definite mechanism is present the resemblances are much less close, the development being hastened in the latter as compared with the former and both being hastened, though in different degrees, as compared with the ontogenetic development.

The discussion of the regulation of the process may be referred to the general discussion of compensatory regulation in the group of Serpulids, as given on p. 76.

3. Probable Phylogenetic Development.

The opercula and branchiæ of the family Serpulidæ furnish as good a case of a morphological series as can be found within the animal kingdom. There are all gradations between species with no modification of the branchiæ up to those with a degree of opercular modification so great that no branchial characters can be made out in the organ. Furthermore, in the ontogeny of the one form studied (Hydroides), in which there is a high degree of modification, each of the two opercula passes through a stage in which it is to all appearances a functional branchia. The paleontological evidence, however, is fragmentary. Our only knowledge is obtained from the calcareous tubes and it is not always possible to decide whether the animal inhabitant was or was not operculate. Tubes evidently belonging to the genus Spirorbis are, however, found as low down as the upper Silurian.

The morphological and ontogenetic evidence leads us to the probable conclusion that the ancestors of the present day operculate Serpulids were non-operculate forms and that the opercula arose in the course of phylogeny by the development of enlargements upon the branchiæ which served to close the opening of the tube in which the animal lived.

Some speculations as to the origin of the asymmetry of the opercula in the Serpulids may be permissible if it is recognized that the course of the probable phylogeny can at present be no more than guessed at. The existence of a morphological series running from forms with no opercular modification of the branchiæ (Protula) through forms with a terminal enlargement at the end of each branchia (Salmacina), others with two equal opercular knobs one on each side of the median line attached to stalks still retaining respiratory pinnules (Filograna), to still others with a large operculum on one side and a small one on the other (Hydroides, etc.) or with one operculum and that lateral in position (Ditrupa, etc.) indicates that the early differentiations of the operculum were symmetrically arranged with respect to the median line.

However, the ontogeny of Hydroides shows an asymmetry from the very first appearance of the opercular modification. Furthermore, the fact that this earliest development always occurs on the left side indicates some correlation between the character of the tube and the position of the organ. In Hydroides, however, there is an irregularity in the coiling of the tube from the very start, so that we get no evidence here of such a relation.

An examination of several Serpulids brings out the following relation between the adult position of the functional operculum and the character of the coils of the tube. A tabulation of the result is given below:

TABLE IX.

Genus	Funct. Operculum	Tube
Spirorbis	Always right	Dextral coil.
Pileolaria	Always left	Sinistral coil.
Ditrupa	Always left	Definite curve. Relation to animal not made out.
Pomatoceros	Always left	Irregular.
Vermilia	Right or left	Irregular.
Hydroides	Right or left	Irregular.
Serpula	Right or left	Irregular.

In the case of the definitely coiled forms Spirorbis and Pileolaria there is a very definite relation between the direction of the coil and the position of the operculum, the operculum being always on the inner side of the opening, *i. e.*, the side next to the concave side of the coil.[1] In Ditrupa this may also be true though the curve of the tube is slight and the relation between body position and the curve was not made out. The supposition of such a relation between opercular position and the curve of the tube is weakened by the fact that the tube lies freely on the sea bottom and, therefore, may lie on either side as far as known. In Pomatoceros triquetroides the operculum is always on the left side though the tube is irregularly coiled.

---

[1] See Caullery et Mesnil ('96).

The functional operculum in the young Hydroides always appears on the left side though later in ontogeny the corresponding branchia of the other side is modified into a rudimentary operculum which possesses the power of developing into a functional one under certain conditions usually connected with injury to the operculum of the opposite side. The tube is irregularly coiled.

The constancy of position of the operculum in Pomatoceros and of the functional one in the larval Hydroides, notwithstanding the irregularity of the coils in the tubes, indicates that these forms may be descended from ancestors with a definitely coiled tube with which, further, the definite position of the operculum was associated. The fruitlessness of such speculations is, however, very evident, especially if an attempt be made to apply the same reasoning to the case of Vermilia multivaricosa, in which the single operculum may be either on the right or on the left side. The coils are here also irregular. Would it be necessary to assume a descent from a definitely (*i. e.*, not irregularly) coiled species which produced both dextral and sinistral, and, therefore, right and left operculate individuals in a manner corresponding with that of some of the species of snails? Speculation from this point of view seems to be of little value.

In connection with this discussion it may be well to state that Vermilia multivaricosa evidently comes under the head of the cases which Conklin ('03) seeks to explain on the basis of a reversal in the polarity of the egg, as there is no mechanism for the reversal of the opercula in post-embryonic development. The two conditions, right and left, in the present case are of practically equal occurrence.

3. *Comparison of Regeneratory, Ontogenetic and Probable Phylogenetic Development.*

As the data obtained from ontogeny have been used in the determination of the probable phylogeny the probability of the course of the phylogeny as given in the present discussion is weakened by the removal of these data though such a removal is made necessary by the character of the comparison. Nevertheless, the morphological series is so complete that there is sufficient ground for the conclusion that the opercula arose in the course of phylogeny as modified branchiæ.

The functional operculum of Hydroides has been shown to arise in ontogeny as a branchia, which later is modified to serve as an operculum. Moreover, in its modification it passes through a series of changes which corresponds very closely with the similar morphological series that may be picked up from different genera of the family. We have thus first, a stage in which the branchia is unmodified (Protula stage); second, a stage with a terminal enlargement on a branchial stalk having the ordinary respiratory filaments still present (Filograna or Apomatus stage); third, a stage in which the respiratory filaments have disappeared and in which the terminal cup has only one row of serrations (Serpula stage). The adult Hydroides condition, with two rows of serrations, is then finally attained as the result of the reversal of the opercula already described. (p. 50.) It is seen that this ontogenetic series corresponds very closely with the probable phylogenetic one.

The rudimentary operculum develops as a branchia which drops off and regenerates a rudimentary structure from the basal stump, so that it also passes through a stage which may be considered to resemble a phylogenetic one.

The *regeneratory development* falls under two heads, the development of the rudimentary operculum from the stump of the old functional or old rudimentary and the later differentiation of this into a functional. The two stages, according to the conditions, follow one another without a break or else there is a period of rest at the rudimentary stage. *The course of regeneration is characterized by a great condensation and directness of the development. There is no trace of a branchial stage and the development of the two rows of processes of the terminal cup does not follow in the ontogenetic order*, that is, the more proximal row (Serpula stage) does not appear before the more distal row (Hydroides stage). The time between the appearance of the two rows is not great *but the more distal row appears before the more proximal one, contrary to the course in ontogeny.* The absence of a branchial stage and the reversal in the order of appearance of the two rows of processes of the cup, therefore, show a wide departure from the ontogenetic development.

The instances so far given include those cases only which involve the regeneration of the opercula alone. When the anterior portion of the body is removed by a transverse cut the regeneration at the anterior end of the posterior piece follows a similar series as regards the development of the opercula, but the manner of regeneration of the branchial circlets shows a striking similarity to that of the first appearance of these organs in ontogeny. In its first stages the circlet consists of a group of four buds, one of which from the first shows its opercular character. The number of these buds recalls very strikingly the number in the ontogeny. The operculum here also, however, shows no trace of the branchial characters so evident in its ontogeny.

The data furnished by the opercula of the Serpulids, therefore, give a fairly close agreement between the ontogenetic stages and the probable phylogenetic ones as determined by the usual criteria. *The regeneratory* development, however, follows a course which may be modified by the character of the operation that leads to the regeneration.

4. *General Discussion of the Facts of Compensatory Regulation in the Opercula of Serpulids.*

The data of both ontogeny and regeneration show that when the opercula have an equal start in development they develop as equal organs. When, however, one has an advantage over the other as regards the time of starting it exerts a retarding influence upon the other and holds the latter in a rudimentary condition. The rudimentary operculum very evidently is either continually held in check by a stimulus from the opposite fully developed operculum or else there is no such continual retarding stimulus, and the removal of the functional produces the positive stimulus for further development, or both these conditions may hold. The manner in which the presence of the large organ on one side acts upon the smaller organ is, of course, not known, but there are various arguments in favor of the view that the control may be a nervous one. The experiments of Wilson ('03) on the nervous control of the process of reversal of the chelæ in Alpheus, my observations on the regeneration of the branchial circlets and tail knobs in Serpulids in connection with the cut ends of the here widely separated nerve cords and other cases, for which reference must be made to a future paper, indicate such a control. (See p. 62.)

The study of the ontogeny of the opercula in Hydroides has cleared up the subject greatly. It is seen that the experimentally obtained reversal is merely an expression of a normal developmental process. The primary functional operculum develops as an asymmetrically placed (left) structure and by virtue of its early development holds in check the embryonic opercular tissue of the opposite side. Later the primary functional operculum drops off, the former rudimentary tissue is no longer retarded, and, therefore, develops to a functional stage when it in turn restricts the now developing bud of the other side. Evidently the experimental reversal is merely an expression of this same process, the artificial removal of the functional operculum by a transverse cut causing the removal of the retardation effects on the opposite bud, and at the same time hastening the formation of the embryonic tissue at the basal suture of its own side, therefore leading to the dropping off of the remanent of its stalk above the suture and leaving room for the new rudimentary to develop as far as the now rapidly developing new functional of the opposite side will allow it. The fact that there is such a retardation effect of the larger organ on the smaller is further indicated by the fact that after transverse section of the body the two opercula which develop are both large and resemble the functional in general character though differing from it in particulars. The same thing is further indicated by the possibility of an explanation on this basis of the complicated results obtained when both opercula are cut off. It has been shown (p. 61) that assuming such an interaction between the opercula as here given we can explain the development of two functionals in one group and the reversal of the opercula in the other by the earlier dropping off of the functional stalk in the former group as compared with the latter, giving the bud of the functional side in the first case an equal chance in the competition with the opposite one, which chance it does not possess in the second case.

IV. THE RATE OF DIFFERENTIATION DURING REGENERATION OF THE OPERCULA IN THE SERPULID, APOMATUS.

The effect of the size of the piece upon the rate of regeneration of the opercula may be very conveniently studied in this species

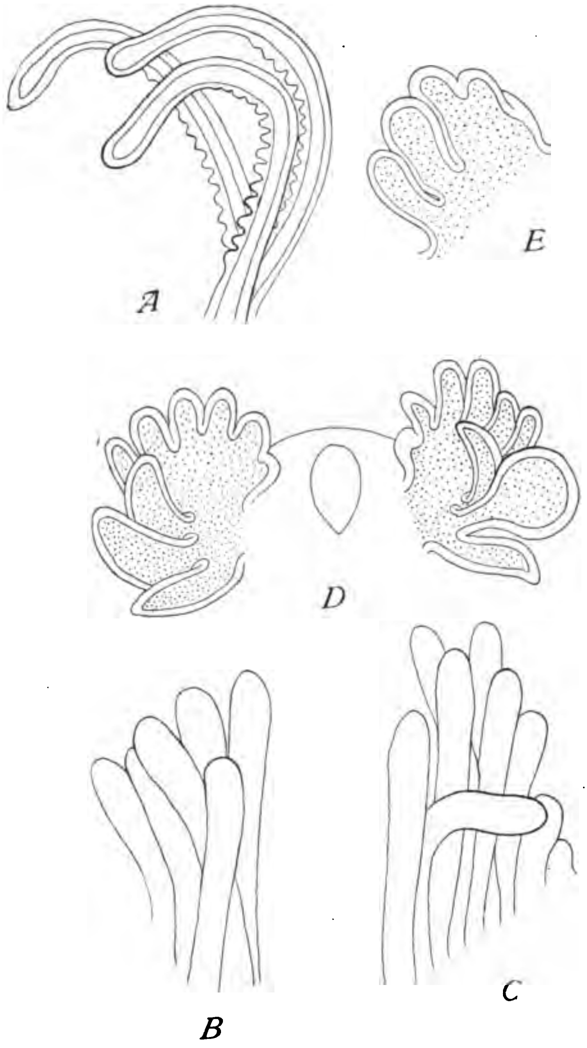


FIG. 25.

Apomatus ampullifera. *A*—Operation: Branchial circlets cast off at breaking joint. Body intact. Figure of three most dorsal branchiæ of right side 9 days after operation. No opercular differentiation (×45). *B*—Operation: Same as in *A*. Figure of regenerating right branchial circlet 6 days after operation. No opercular differentiation. *C*—Left branchial circlet of same (×62). *D*—Operation: Branchial circlets thrown off at breaking joint. Body posterior to third thoracic segment removed. Eight days after operation. Note opercular differentiation (×62). *E*—Operation: Same as *D*, except that thoracic cut is at second segment. Figure shows the regenerating left branchial circlet 6 days after operation. Note beginning of opercular enlargement.

because there is a very definite line of cleavage along which the break always takes place, and similar materials may be assumed to exist at the regenerating surface at the time of the operation in all individuals of a set of experiments. If, therefore, the body is cut in two at various levels and the branchiæ are thrown off at the "breaking joint" any differences in the regeneration of the branchiæ and opercula may be considered as due to differences in the posterior body operations.

In the *first* lot of Apomatus the branchiæ were removed in the manner mentioned but there was no operation on the body. In the *second* lot the body was cut between the third and fourth thoracic segments in addition to the removal of the branchiæ, and in a *third* lot the thoracic cut was made between the first and second segments. The last two operations in a great number of the cases naturally caused the death of the animals, but in general a very interesting result was obtained. In the cases where the body was cut in two in the thorax as mentioned *the opercular differentiation appeared much earlier than in those in which the body was intact.*

When the body is uninjured except for the removal of the branchial circlets the branchial buds to the number of eight or nine on each side appear simultaneously or nearly so, although there is a slight gradation in size from dorsal to ventral edge of the branchial ridge from the beginning. The few remaining buds to be developed are added from the ventral edge. These bud-like processes increase rapidly in length and soon appear as long slender filiform processes which usually take on the secondary pinnules before the appearance of the first traces of opercular differentiation. The opercular differentiation then appears as a vesicular enlargement in the next to the dorsal branchia on each side.

In the two-segment and four-segment thoracic pieces, left by the thoracic cuts in lots two and three, the development does not follow this course. The opercular bud is from the start very evidently different from the others, being larger and more spherical than the branchial buds proper. This is well shown in the figures. (Figs. 25 and 26.) The branchiæ in this case, however, are also thicker and shorter than the corresponding ones where the body remains intact. It is very evident that while in the one case where the body is intact the operculum passes through a distinct branchial stage before showing even a beginning of an opercular knob, in the other case where only the anterior two or four segments remain the operculum appears as such from the start.

Unfortunately the animals in the last two lots did not live long enough to show whether or no the final outcome would have been the same in the two cases. In fact none of them showed any pinnules on the branchiæ at the time of death. Notwithstanding these limitations it is evident that we have a definite acceleration of the rate of differentiation of the opercula.

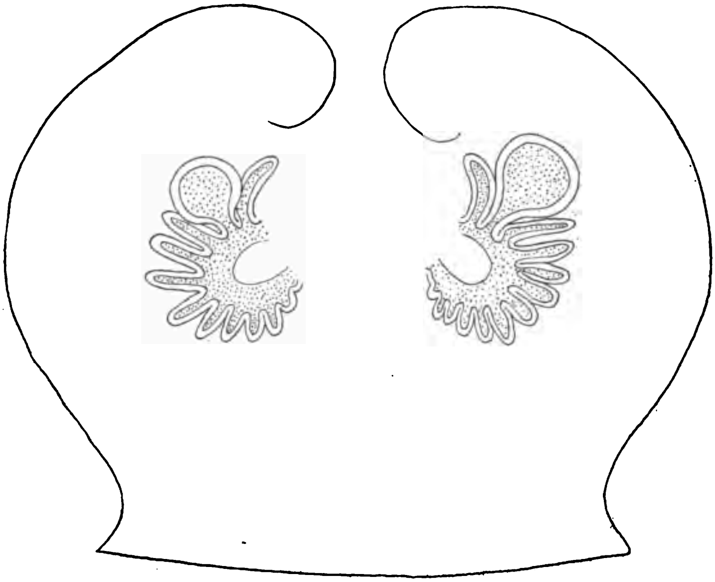


Fig. 26.

Apomatus ampullifera. Regenerating branchial circlets. Nine days after operation (×40). Operation: Autotomy of both branchial circlets at breaking joint and removal of body posterior to second thoracic segment. Note pronounced opercular differentiation on both sides.

Two probable factors may be mentioned as concerned in the bringing on of the acceleration. 1. The shock of the transverse division of the body may lead to such an increase in rapidity of differentiation. 2. The small size of the piece itself may directly influence the process and bring about such a differentiation. This action may result because of the difference in the interactions of the organs in the one case as compared with the other.

V. REGULATION OF THE RATE OF GROWTH AND NATURE OF DIFFERENTIATION DURING REGENERATION OF THE CHELAE OF GELASIMUS AND ALPHEUS.

1. *Introduction.*

The general problem to be taken up in the experiments on the chelæ of the two Decapod Crustaceans mentioned corresponds with that already given for the Serpulids. The interactions of the two chelæ naturally constitutes the principal point of study. Likewise the influence of the removal of one or both chelæ upon the rate of moulting of the animals will be discussed and some further incidental points will be touched.

In Gelasimus pugilator the two chelæ are of nearly the same size and character in the female but differ widely in the male. In Alpheus dentipes the chelæ differ both in size and character in both male and female.

2. *Gelasimus Pugilator.*

In Gelasimus the male has one of the two chelæ enormously developed. This large chela is nearly equally distributed between right and left sides in a group of individuals taken at random. In the female the two chelæ are small and equal in size. The animals readily autotomize their legs if a needle is inserted between two of the joints distal to the "breaking joint" so as to touch the nerve. In the following experiments the animals were made to throw off their chelæ in the way mentioned. They were kept in glass dishes with just sufficient water to keep them moist and fed with bits of the horse-mussel, Mytilus. Under these conditions they lived very well, though unfortunately the growth of the new legs was extremely slow and the experiments could not be completed as satisfactorily as was wished.

1. Experiments on Males.

a. Large Chela Alone Removed. The first object of the work was to determine whether reversal of the character of that of Hydroides takes place in these forms. The large chela was autotomized in the manner already indicated. In the great majority of the cases the animals lived through the 62 days after the operation, but in only a few did a moult take place so that the results are not entirely satisfactory. Twenty specimens, ten with the large chela on the right side, and ten with it on the left, were treated in this way. Five specimens had moulted at the end of 62 days after the operation when the experiment was closed. The first one moulted 54 days after the operation and in this the regenerated chela (= former large one) was as yet smaller than the other (= former small one). The old small one had no pronounced change as a result of the moult. In the four other specimens, one of which moulted 59 and the other three 62 days after the operation, the new regenerated chela was in each case larger than the opposite old one, though it had not as yet attained the full size and characteristics of the typical large one.

It may be safely concluded from the above observation that no reversal of the chelæ in the sense of the reversal of opercula in Hydroides takes place in the males of Gelasimus after removal of the large chela, for it seems evident that in the first case mentioned, where the regenerated chela was as yet smaller than the old small one of the opposite side, it had not yet reached its full growth. In further support of this view is the fact that no pronounced change in the old chela was noticed.

b. Small Chela Alone Removed. The following results were obtained when the small chela alone was removed: Only four of the ten specimens moulted before the end of the 62 days, constituting the limit of the experiments. In all of these the newly regenerated chelæ were much smaller than the opposite large ones and approached in character the ordinary small chelæ. The four specimens mentioned moulted, respectively, 48, 61, 61 and 62 days after the operation. Therefore, here also there is no reversal of the chelæ.

c. Both Chelæ Removed. In this set of experiments both chelæ were autotomized. Ten specimens were kept for 62 days and eighteen for 42 days. Seven of the former moulted and showed the characters of the regenerated chelæ. In each of the seven a large chela was regenerated in place of the former large one and a small chela in place of the former small one. There was no reversal. The chelæ after the first moult did not of course as yet have the full size of the old ones but the difference in size was very evident.

In one of the seven cases mentioned here as having moulted so as to show the characters of the regenerated chelæ one specimen showed an abnormality in that the smaller regenerated chela had two pinchers at its end. This case will be described in a separate note at another time.[1]

d. Two normal male specimens kept in glass dishes for 62 days did not moult or show any changes. They were fed on fragments of Mytilus in the same manner as the others.

2. Experiments on Females.

a. Removal of One Chela (right or left). Two of the six specimens moulted before the completion of the experiment. In one of these the regenerated chela was a trifle smaller than the opposite one. In the other the two chelæ, the old and the regenerated one, were nearly equal in size after the moult. One of these specimens moulted 56 days, the other 62 days after the operation.

b. Removal of Both Chelæ. Only three specimens were operated on in this way. All had moulted within 46 days after the operation. The new animals regenerated two new and equal chelæ. The regenerated structures, therefore, repeat the character of the removed appendages.

3. The Rate of Regeneration and of Moulting After the Operation. (Male and Female.)

The data show very plainly that the moulting takes place sooner in the cases where both of the chelæ are removed than in the cases where only one or none are removed. In fact all three members of the female set with both chelæ removed moulted before any of those with only one chela removed had done so. It does not seem possible that the matter of accident can come in here as there are too many cases both as regards this point and as regards other related ones.

A general comparison in both males and females of the cases in which both chelæ were removed with those in which only one or none were removed bring out an interesting result.

1. *Time of moulting. The specimens with both chelæ removed moulted sooner than those with only one chela removed.*

2. *The regenerating buds in the specimens where both chelæ had been removed were in general larger than the corresponding buds*

---

[1] Biol. Bull., '05.

where only one chela was removed. This statement is of course not a definite quantitative one on account of the difficulty in estimating relative sizes, but becomes interesting in connection with the following results on Alpheus (p. 85, *ff.*) and the former cases already described.

The results, therefore, indicate that where two chelæ are removed the time of moulting is hastened and the rate of regeneration of each chela is increased as compared with the cases where only one chela is removed.

4. Rate of Moulting after Removal of One or Both Eyestalks. (Male and Female.)

Three sets of experiments were performed.

a. In one set of three females the right eyestalk was cut off near its base. Pigment very soon collected near the cut end so that the region assumed a color much darker than the normal eye color. A similar change took place in all the other experiments after the cutting of the eyestalk.

b. In another set there were three males, one with the large chela on the left and two with it on the right side. The right eyestalk was cut off near its base. The first noticeable change as in the last set was a collection of very dense pigment at the cut surface. All three of the specimens moulted before the close of the experiment, one 56 days, another 58 days and the third 61 days after the operation. At moulting the dark pigment of the cut surface disappeared and the end of the new eyestalk was rounded and resembled the old eyestalk except that it was shorter.

c. In a third set of four males and one female both eyestalks were cut off near their bases. Two of the specimens lived for more than a few days. One moulted 14 days after the operation but was found dead and broken after the moult. In the other one the cut surfaces of the eyestalks were black with pigment at this time and also five days later, 19 days after the operation. This specimen moulted 23 days after the operation, at which time the eyestalk ends were rounded but there was no appreciable increase in eyestalk length. The very dark pigment disappeared with the moult as in the last series. The animal died 40 days after the operation.

d. Conclusion. In the specimens with an eyestalk operation the time of moulting was hastened as compared with unoperated specimens. In the cases where both eyestalks were removed the moults came sooner (14 and 23 days) than when only one was removed (56, 58 and 61 days). Here again, therefore, there is a hastening of the physiological process in the cases of a greater disturbance as compared with those of a lesser disturbance of the organism.

3. *Alpheus Dentipes.*

1. Introduction and Review of Former Work.

The reversal of the cutting and snapping chelæ of Alpheus has been demonstrated and the process studied by Przibram ('01,'02) and recently by Wilson ('03) and Brues ('03, '04). It has been shown that when the snapping claw is removed, the cutting chela of the opposite side is differentiated into a snapping chela, while in place of the removed snapping chela a new cutting chela is developed. When the cutting chela alone is removed there is no reversal, a new cutting chela developing in place of the old. When both chelæ are removed there is again no reversal, a new snapping chela being regenerated in place of the old snapping and a new cutting chela in place of the old cutting one. In the latter case Przibram, working on A. dentipes and A. platyrrhynchus mentions the fact that the newly regenerated cutting chela is relatively larger as compared with the snapping chela than in the animal before the operation. Wilson's result on the Beaufort, N. C., species (H. heterochelis) agrees with that of Przibram except that in the case where both chelæ were removed the new cutting chela does not approach the new snapping chela as nearly as in the species upon which Przibram worked. Wilson further added the interesting result that when the snapping chela is removed and the nerve leading to the cutting chela is cut below the breaking joint

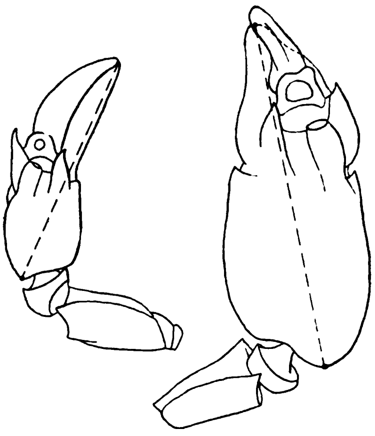


Fig. 27.

Chelæ of Alpheus dentipes (×4). Left-hand figure: cutting chela. Right-hand figure: snapping chela. Dotted lines represent the lengths measured (see text, p. 91).

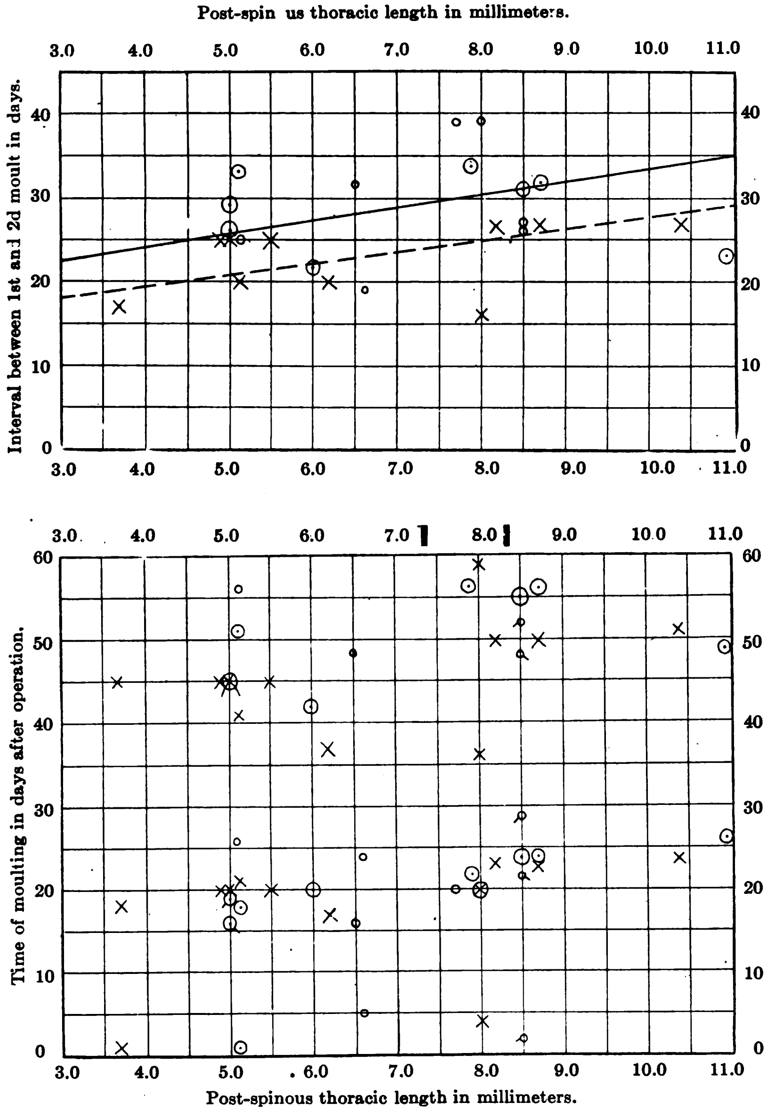


Fig. 28.

Alpheus dentipes. Lower data—Time of moult in days after operation. Upper data—Interval between first and second moult in days. Abscissæ are post-spinous thoracic lengths. ○ = Cutting chela (Cu) alone removed. ⊙ = Snapping chela (Sn) alone removed. × = Both chelæ (Cu + Sn) removed. In the upper data the upper line is drawn to fit the first two groups of cases (one chela alone removed) and the lower line to fit the last group (both chelæ removed).

"the reversal in some cases at least does not take place or is incomplete." Brues (Wilson, '03, p. 210) adds the interesting fact that in A. heterochelis the nerves supplying the two chelæ and the ganglionic centers from which they proceed do not differ perceptibly in size.

2. The Data.

The experiments about to be described in the present section of the paper were performed at the Naples Zoölogical Station in the winter of 1902–03. They confirm the general facts of reversal of the chelæ as given above. Their main object, however, was the determination *first*, of the effect of the removal of one or both chelæ upon the rate of moulting of the animal, and, *second*, of the influence of the presence or absence of the opposite chela upon the rate of regeneration of a chela.

a. The Influence of the Removal of One or Both Chelæ upon the Rate of Moulting of the Animal. Three sets of specimens were operated on. In one set (*Sn*) the snapping chela alone was removed. In a second (*Cu*) the cutting chela alone was removed. In a third (*Sn* + *Cu*) both snapping and cutting chelæ were removed. The animals were kept in isolated dishes for 59 days after the operation and were fed either every day or every other day on small pieces of fresh fish meat. Without taking into account the cases where the legs were accidentally autotomized a second time during the experiment, or in which other disturbances occurred, we have the following relation between the time of moulting and the post-spinous thoracic length of the animals without reference to the character of the operation: On the coördinate paper (Fig. 28, p. 86) the abscissæ represent the thoracic lengths in millimeters and the vertical columns (ordinates) the days after the operation when moulting occurred. The animals were killed 59 days after the operation. The data from the first set (*Cu*) are represented by the symbol ○, those of the second set (*Sn*) by the symbol ⊙ and of the third set (*Sn* + *Cu*) by ×. It will be seen that in general the moulting interval increases with the size of the animal as represented by the thoracic length.

On pp. 88 and 89 the data are put in Tables X, XI and XII, each of the sets being placed by itself. The interval of time in days between the first moult and the second moult is put down in a separate column. Upon averaging this interval in the three sets separately it is found that *the interval decreases from 29.6 days for the Cu set and 28.7 days for the Sn set to 22.9 days for the Sn + Cu set.* This result is represented on coördinate paper on p. 86, Fig. 28.

When two chelæ are removed there is, therefore, a shortening of the period between the first two moults as compared with the cases where only one chela is removed. It will be seen that there is only a slight difference between the moult period in the two single chela cases. This result agrees perfectly with that obtained for the time of appearance of the first moult in Gelasimus, where it was found (p. 83, *ffl.*) that the specimens with both chelæ removed in every case except one moulted before those with only one chela removed.

TABLE X. *Alpheus dentipes. Time of Moulting. Cutting Chela (R or L) Removed.*

Cat. No.	Thoracic Length.	Date of Operation.	1st moult.	2d moult.	3d moult.	Interval 1st–2d.
		1903				
562	6.6	I/8	5	24	—	19
565	8.5	I/8	2	29	52	27
569	8.0	I/8	20	—	—	39+
573	8.5	I/9	22	48	—	26
576	6.5	I/9	16	48	—	32
579	5.1	I/9	1	26	56	25
582	7.7	I/9	20	—	—	39+
					Av. =	29.6

The greater disturbance of the normal condition of the animal here again causes greater activity as regards the moulting period. Starting with the smallest disturbance and going upward we have a series ranging, respectively, through (1) cutting chela removed, through (2) snapping chela removed, to (3) both chelæ removed. Correspondingly, the interval between the first and second moult decreases from 29.6 days, through 28.7 days to 22.9 days for the cases named.

TABLE XI. *Alpheus dentipes Time of Moulting. Snapping Chela (R or L) Removed.*

Cat. No.	Thoracic Length.	Date of Operation.	1st moult.	2d moult.	3d moult.	Interval 1st–2d.
561	8.7	I/7	24	56	—	32
563	7.9	I/8	22	56	—	34
575	8.5	I/9	24	55	—	31
578	5.0	I/9	19	45	—	26
581	5.0	I/9	16	45	—	29
617	6.0	I/7	20	42	—	22
619	11.4	I/7	26	49	—	23
620	5.1	I/7	18	51	—	33
					Av. =	28.7

TABLE XII. *Alpheus dentipes. Time of Moulting. Both Chelæ Removed.*

Cat. No.	Thoracic Length.	Date of Operation.	1st moult.	2d moult.	3d moult.	4th moult.	Interval 1st–2d.
		1903					
566	6.2	I/8	17	37	—	—	20
567	8.7	I/8	23	50	—	—	27
571	10.4	I/9	24	51	—	—	27
574	8.2	I/9	23	50	—	—	27
577	5.5	I/9	20	45	—	—	25
580	4.9	I/9	20	45	—	—	25
583	5.0	I/9	20	45	—	—	25
584	5.1	I/9	21	41	—	—	20
585	3.7	I/9	1	18	45	—	17
618	8.0	I/7	4	20	36	59	16
						Av. =	22.9

SUMMARY OF TABLES X, XI, XII. *Comparison of Interval Between First and Second Moults.*

Cu Removed = 29.6 days. (Av. of 7 cases.)
Sn Removed = 28.7 days. (Av. of 8 cases.)
Cu+*Sn* Removed = 22.9 days. (Av. of 10 cases.)

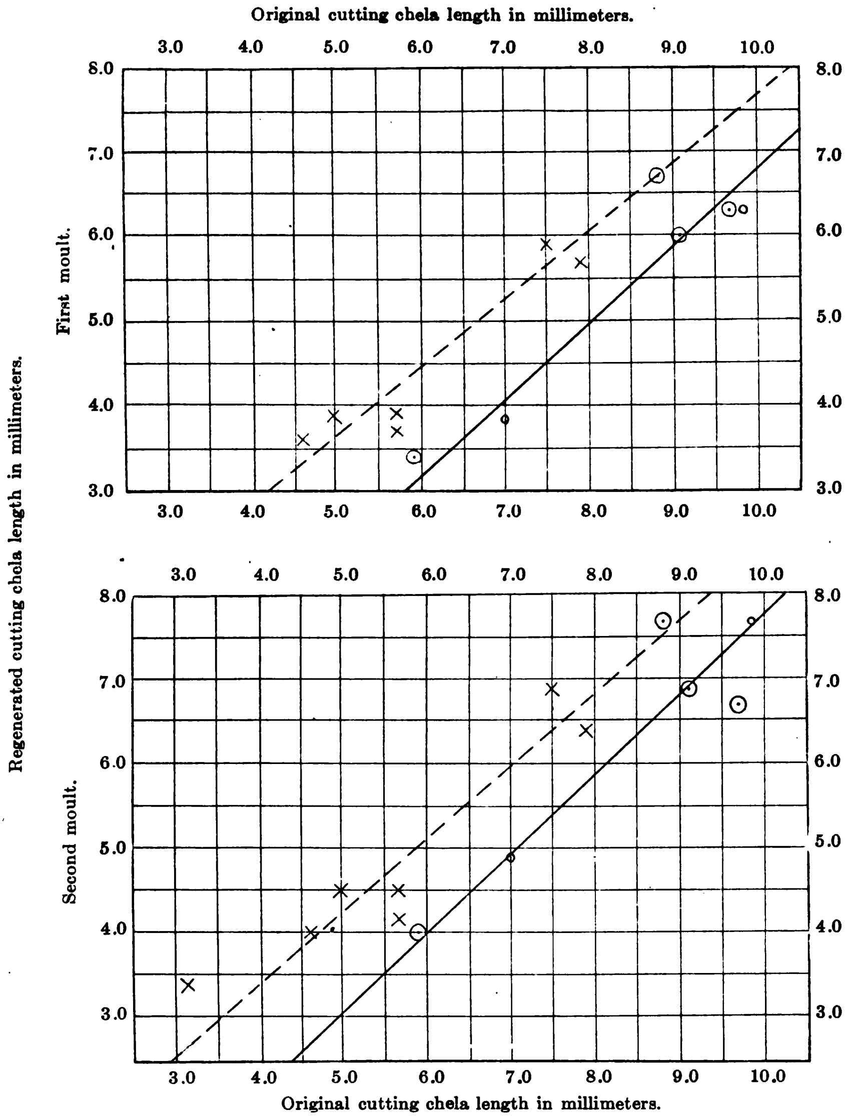


FIG. 29.

Alpheus dentipes. Lengths of regenerating cutting chelæ at the end of the first and second moults. Ordinates = Regenerated cutting chela lengths in mm. Abscissæ = Original cutting chela lengths. ○ = Cutting chela (Cu) alone removed. ⊙ = Snapping chela (Sn) alone removed. × = Both chelæ (Cu + Sn) removed. The broken lines fit the last group (both chelæ removed) and the unbroken lines fit the first two groups (one chela alone removed).

b. The Influence of the Presence or Absence of the Opposite Chela upon the Rate of Regeneration of a Removed Chela. A comparison was made of the regenerated lengths of the cutting chela in cases where only one chela was removed with cases where both chelæ were thrown off.

When the cutting chela alone was autotomized a new cutting chela was regenerated in its place. When the snapping chela alone was removed there was a reversal and the old cutting chela was differentiated into the new large snapping chela, while in place of the removed snapping chela a typical cutting chela was developed. In the second case this new cutting chela is the one taken in our measurements. In a third case both chelæ were thrown off, a new cutting chela regenerating in place of the old cutting chela and a new snapping chela in place of the old snapping one. The lengths are taken from the moulted casts of the animal, the original length being taken from the cast of the first moult, the first moult condition from the cast of the second moult, etc. The final measurements are taken from the alcoholic specimens of the animals killed 57 days in each case after the operation. The lengths measured in the data about to be described are the greatest lengths of the cutting chela, *i, e.*, the distances from the tip of the pincher process of the fourth podomere to the farthest corner of the base. (Fig. 27.) The chelæ or their casts were in every case drawn carefully to scale by the aid of a camera lucida and the measurements are taken from these drawings. Out of 29 specimens kept for 59 days only 12 can serve for first moult data and 13 for second moult data. The others are not valid because of the death or escape of the animal or the accidental secondary autotomy of one or both of its appendages.

The relation of the regenerated chela lengths to the original lengths is shown on coördinate paper (Fig. 29) for both the first and the second moult. The number of individual cases is small, but it is evident that the regenerated lengths of the cutting chela in the cases where both chelæ were removed have a distinct advantage over the others. This is especially clear for the first moult.

The relation comes out very clearly when we take the ratio between the regenerated cutting chela length and the original length in that specimen as our basis for comparison, for we see that the regenerated length increases as we go from small original lengths to large original lengths. As the cases given on the coördinate paper (p. 90, Fig. 29) show, the correlation is not a perfect one, *i. e.*, it is positive but equal to slightly less than one. The results of such a comparison are given for both the first and the second moults in Tables XIII–XVI.

TABLE XIII. *Alpheus dentipes. Length of Regenerated Cutting Chela. Cutting Chela Removed.*

Cat. No.	Original Cu. Lg.	Reg. Cu. Lg. 1st moult.	$\frac{\text{Regen. Cu.}}{\text{Orig. Cu.}} \times 100$	Reg. Cu. Lg. 2d moult.	$\frac{\text{Reg. Lg.}}{\text{Orig. Lg.}} \times 100$
573	7.0	3.8	54.3	4.9	70.0
576	9.8	6.3	64.3	7.7	78.6
—	—	—	59.3	—	74.3

TABLE XIV. *Alpheus dentipes. Length of Regenerated Cutting Chela. Snapping Chela Removed.*

Cat. No.	Original Cu. Lg.	Reg. Cu. Lg. 1st moult.	$\frac{\text{Regen. Cu.}}{\text{Orig. Cu.}} \times 100$	Reg. Cu. Lg. 2d moult.	$\frac{\text{Reg. Lg.}}{\text{Orig. Lg.}} \times 100$
561	8.8	6.7	76.1	7.7	87.8
563	9.1	6.0	65.9	6.9	75.8
575	9.7	6.3	64.9	6.7	69.1
578	5.9	3.4	57.6	4.0	67.8
—	—	—	66.1	—	75.1

The tables show that $Cu + Sn$ has a very distinct advantage over Cu alone or Sn alone. This advantage amounts to 19.1 per cent for the first moult and 16.5 per cent for the second moult. Just after the first moult the cutting chela regenerated from the breaking joint surface of what was formerly the snapping claw has a distinct advantage over the cutting chela regenerated from a removed cutting chela but this advantage is nearly overcome at the time of the second moult.

Table XV. *Alpheus dentipes. Length of Regenerated Cutting Chela. Both Chelæ Removed.*

Cat. No.	Original Cu. Lg.	Reg. Cu. Lg. 1st moult.	$\frac{\text{Regen. Lg.}}{\text{Orig. Lg.}} \times 100$	Reg. Cu. Lg. 2d moult.	$\frac{\text{Reg. Lg.}}{\text{Orig. Lg.}} \times 100$
566	5.7	3.7	64.9	4.2	73.7
567	7.5	6.4	85.3	6.9	92.0
574	7.9	5.7	72.2	6.4	81.0
577	5.7	3.9	68.4	4.5	78.9
580	3.2	—	—	3.4	106.2
583	4.6	3.6	78.3	4.0	86.9
584	5.0	3.9	78.0	4.5	90.0
—	—	—	74.5	—	87.0

Table XVI. *Summary of Tables XIII, XIV and XV.*

		First Moult.	Second Moult.
Original data. $\frac{\text{Regenerated Lg.}}{\text{Original Lg.}} \times 100$ ...	Cu	59.3	74.3
	Sn	66.1	75.1
	Cu + Sn	74.5	87.0
Comparisons. Absolute difference	(Cu + Sn) − Cu	+15.2	+12.7
	(Cu + Sn) − Sn	+ 8.4	+11.9
	Sn − Cu	+ 6.8	+ .8
Per cent of increase	$\frac{(\text{Cu} + \text{Sn}) - \text{Cu}}{\text{Cu}}$	+25.6	+17.1
	$\frac{(\text{Cu} + \text{Sn}) - \text{Sn}}{\text{Sn}}$	+12.7	+15.8
	$\frac{\text{Sn} - \text{Cu}}{\text{Cu}}$	+11.5	+ 1.1

The general result is clear. The regenerated cutting chela is larger in the case where two chelæ are regenerating than where one alone is to be replaced. This result is emphasized by the fact that the time between the first two moults is shorter in the specimens with two chelæ removed than in those with only one gone. (See p. 87.)

3. Discussion.

The significance of the data may be emphasized by bringing them out in two ways, one of which lays special stress upon the fact of removal of a certain organ or organs and the necessity of a certain amount of regulation in restoring the normal form, and the other of which emphasizes rather the interactions of the two chelæ as parts of a system normally stable at the condition with a large snapping chela on one side and a small cutting chela on the other.

The first point of view in which the total necessary amount of regulation is compared with the rate of regeneration of a part of the whole will first be taken up. The three series of experiments may be compared in the following way:

1. With the cutting chela alone gone the animal has merely to accomplish the regeneration of this organ in order to regain its normal condition.
2. With the snapping chela alone gone the animal has not only to regenerate a cutting chela in place of the old snapping one but also to differentiate the tissue of the old cutting chela into the new snapping chela.
3. With both chelæ gone the animal has not only to regenerate a new cutting chela in place of the old one but also to regenerate a new snapping chela.

Taking the ratios given in Table XVI, p. 93, we see that in the first case where the least work is to be accomplished, at the end of the first moult the cutting chela has regained but 59.3 per cent of its original size, while in the second case it has reached 66.1 per cent, and in the third case 74.5 per cent of its original size. At the end of the second moult likewise we have, respectively, 74.3 75.1 and 87.0 per cent for the three cases in question.

Therefore, the amount of actual regeneration accomplished in the cutting chela is greater the greater the amount of other work of a similar character to be accomplished at the same time.

But if we consider the matter from the second point of view, namely, of the influence of the presence of another similar and opposite organ upon the regenerating tissue, the apparent anomaly of the case is cleared up to a great extent. For it is seen that in the first case (*Cu* alone removed) we have an uninjured opposite large snapping chela to retard the growth of the regenerating cutting chela. In the second case (*Sn* alone removed) we have as the retarding agent at the beginning merely the smaller *Cu* chela which, however, gradually undergoes the changes in size and character leading up to the large snapping chela. Finally, in the third case (*Cu* + *Sn* removed) we have at the beginning no retarding agent, though gradually the snapping chela develops from this point.

Expressing this in concise form we have the following relation referring to the *retarding agent* as indicated by the size and complexity of differentiation of the chela situated on the side opposite to the developing cutting chela:

The *snapping chela* in the group where the cutting chela alone is removed is greater than the *cutting chela developing into a snapping chela*, as in the group where the snapping chela alone is removed and this in turn is greater than the retarding agent where both chelæ are removed, and which amounts to *zero at the beginning with a gradual development up to a snapping chela condition.*

And referring to the corresponding regenerated lengths of the cutting chela we have:

The amount of regeneration of the cutting chela in the first group (*Cu* alone removed) is less than the amount of regeneration of the cutting chela in the second group (*Sn* alone removed), and this in turn is less than the amount of regeneration of the cutting chela in the third group where both chelæ are removed.

In graphic form this may be represented as follows:

Snapping chela [as in Cu group] >
Cutting chela (→ snapping chela) [as in Sn group] >
Zero (→ snapping chela) [as in (Cu + Sn) group].

Correspondingly,

Amount of regeneration of cutting chela in Cu group
< Amount of regeneration of cutting chela in Sn group
< Amount of regeneration of cutting chela in Cu + Sn group.

Evidently the differences between the retarding influences of the three members are greatest near the beginning of the experiments, *i. e.*, immediately after the operation, and gradually decrease as we go away from this point. Correspondingly, the results show a greater comparative difference in regenerated material at the end of the first moult than at the end of the second moult.

This result agrees very well with the experiments on the fiddler-crab Gelasimus (p. 81) and on the brittle-star Ophioglypha, (p. 7).

As moulting involves not only an increase in bulk of the animal but also a complicated degree of differentiation of materials before it can be accomplished, we may likewise compare the acceleration of moulting in Alpheus and Gelasimus with the acceleration of the rate of differentiation of the opercula in Apomatus when the posterior region of the body is also removed as compared with the cases where this region is uninjured.

GENERAL DISCUSSION.

The following discussion does not serve as a summary of the data of the preceding sections. For this the reader must be referred to the summaries of the individual sections which are complete entities in themselves. It is the writer's purpose in the general discussion to show the manner in which the various data, at first sight seeming to have little in common, can be brought under a common point of view.

In the introduction it was stated that the standpoint of the present paper would be the consideration of the organism as a system made up of mutually interacting parts, the relations of which were to be studied by noting the disturbances produced as a result of the removal of one or more of the parts.

In the paper on the dimensional relations of the members of compound leaves the relations of the parts of a system were studied in which the removal of one member was not followed by its regeneration but resulted in changes in size and position of the remainder. The chief reactions were the following: In the five-leaved forms in which an asymmetrically placed leaflet was removed the other four leaflets tended to rotate to a position such that the new system was a symmetrical four-leaved one. Likewise in the three-leaved system after removal of one of the asymmetrically placed leaflets the two remaining leaflets tended to take up a position so as to form a symmetrical two-leaved system. From the position reactions it is evident that the parts of the normal compound leaf are exerting a continual influence upon each other which when resolved into its resultants gives rise to a configuration very definite for a given species. The removal of one of the parts changes the whole system of reactions, and we have a tendency toward the formation of a new stable symmetrical system, with one less leaflet than the original number, the completeness of the new symmetry being only limited by the rigidity of the leaflets.

In Ophioglypha we have a radial system in which the removed arms are regenerated. The experiments on the rate of regeneration bring out the presence of an unsuspected interaction between the arms which must naturally be correlated with some interaction present in the perfect, unmutilated animal. The data of the experiments show that (leaving out of consideration the cases where all five arms are removed and which cannot be used because of the early death of the animals) the rate of regeneration of an arm is greater the greater the number of other arms removed at the same time. This indicates an interesting interaction of the arms upon each other for the presence of unremoved arms seems to retard the rate at which the removed ones are regenerated, for it is not probable that the increase in rate in the one case is due entirely to the increase in stimulus to regeneration produced by the added injuries.

The two members of a pair of appendages in bilateral animals have been shown by the present experiments to have a profound influence upon each other.

In those Serpulids, for example, which have one large functional and one small rudimentary operculum it has been shown that either organ originally has the potentiality of developing into a functional operculum, which is to be developed in this way, depending upon the matter of an early start. When one side gets a start over the other the development of the latter is restricted to a rudimentary stage, while the former develops to a full functional size. Also, when the functional operculum is removed its restricting influence being removed at the same time, the rudimentary operculum immediately develops into a functional one, which in turn restricts the developing new bud of the other side. When both develop at the same time, as from the anterior cut surface of the thorax, two functional opercula are formed.

Similarly, in the Decapod Crustaceans the two chelæ have a profound influence upon each other.

In Alpheus there are two chelæ, one a larger "snapping" chela and the other a smaller "cutting" chela. The snapping chela seems to hold the cutting chela in check, for as soon as the former is thrown off the cutting chela changes over to the snapping chela by a qualitative and quantitative change combined. The new organ regenerating in place of the old snapping chela comes into a system no longer relatively like the old, so that the interaction of parts forces it into a different niche in the new order of things. The reversal, here as in the Serpulids, is easily understood in the way mentioned, if we consider the systems as asymmetrical interacting systems such that the removal of one part can lead only to the development of a certain definite structure. The removal of the organ (functional operculum or snapping chela) brings about an instability in the system which because of the reactions between the parts tends to assume the condition of a new *stable* system. This new system reacts now in a different way on the regenerating organ, causing it to develop into a different structure. The readjustment in the old material and in the regenerating material is further complicated by the fact that both processes go on at the same time, the final outcome being the resultant of both.

In Alpheus as also in Gelasimus we have an interesting relation between the two chelæ, in that when both are removed the rate of regeneration is greater in each than when one alone is removed. Evidently this comes under the Ophioglypha relation that the presence of an unremoved organ retards the rate of regeneration of a removed one. Likewise if we consider the cutting chela of Alpheus as a stage in the development of the snapping chela (Wilson, '03) and the rudimentary operculum as a stage in the development of the functional, we can say that the presence of the larger organ retards the differentiation of the smaller one. This comes into relation with the series of experiments on the rate of differentiation of the regenerating opercula in Apomatus, in which it was found that the absence of the posterior region of the body back of the second or fourth thoracic segment accelerates the rate of differentiation of the regenerating opercula.

From the point of view of the retarding influence exerted by one organ upon another the data that have been brought out in the present paper may be collected in the following concise form:

1. *Ophioglypha.* Arms *a*, *b*, *c*, *d*, *e*.

a_1 = Rate of regeneration of an arm when it alone is cut off.
a_2 = Rate of regeneration of an arm when two arms are cut off, etc.
r_{a_1} = Retardation as result of influence of remaining arms on a_1, etc.

Then

$$a_5 = a_4 + r_{a_4} = a_3 + r_{a_3} = a_2 + r_{a_2} = a_1 + r_{a_1}$$

arms remaining =	none,		*e*,		*d* and *e*,		*c*, *d* and *e*,		*b*, *c*, *d* and *e*.
In the above	a_5	$>$	a_4	$>$	a_3	$>$	a_2	$>$	a_1
and	0	$<$	r_{a_4}	$<$	r_{a_3}	$<$	r_{a_2}	$<$	r_{a_1}.

2. *Hydroides.* Opercula, $OF_{R \text{ or } L}$ and $OR_{L \text{ or } R}$.

$OF_{R \text{ or } L}$ = Functional operculum, right or left.
$OR_{L \text{ or } R}$ = Rudimentary operculum, left or right.

Taking for the sake of simplicity the opercula as OF_R and OR_L we have:

Operation.		*Result.*	*Explanation on "Retardation" Theory.*
(1) Both off. (thoracic cut)	=	$OF_R + OF_L$	No retardation of one by the other. Therefore both reach full development.
(2) Functional off.	=	$OR_R + OF_L$	Reversal of opercula. Release of normal retardation of old rudimentary and the presence of a new retardation influence upon the new rudimentary. ∴ a reversal.
(3) Rudimentary off.	=	$OF_R + OR_L$	Full action of old retardation influence upon the new bud and therefore no change.

3. *Gelasimus.* Chelæ CR, CL. Asymmetry in ♂ only.

In ♂ one chela is considerably larger than the other, but the condition is fixed and cannot be reversed as can the opercula of Hydroides.

In ♀ the two chelæ are equal.

CR + CL, as compared with CR alone or CL alone, shows an acceleration of the time of moult and has each chela bud larger than the single bud of the latter.

4. *Alpheus.* Chelæ CR, CL. Asymmetry in both ♂ and ♀.

The basal ganglia controlling the chelæ are similar on the two sides (Brues).

(1.) Reversal takes place as in Hydroides.

(2.) Relations of time of moult and size of regenerated structure similar to those of Gelasimus.

5. *Apomatus.* Serpulid. Opercula $\mathrm{OF}_{\mathrm{R\ or\ L}}$ and $\mathrm{OR}_{\mathrm{L\ or\ R}}$. (See Fig. 23D.)

Operation. Removal of two branchial circlets + opercula at basal suture. Uniform in all experiments.

(1) In one set of experiments body of animal is kept entire.

(2) In other set of experiments body of animal back of second or fourth thoracic segment is removed.

Result:

Rate of differentiation of new opercula in (1) $<$ Rate of differentiation of new opercula in (2).

because

Retarding influence of whole body (1) $>$ Retarding influence of anterior two or four thoracic segments (2).

The superficiality of the attempted explanation of the data is very apparent, but it is from this point of view that the analysis of the problems must be attacked. That the factors which it is attempted to isolate are real factors in normal development is shown by the physiological reversal of the opercula which takes place in the ontogeny of Hydroides, a process in all ways similar to the reversal after artificial section of the stalk of the functional operculum. From such a point of view the present analysis may be of value as affording a basis for further experimentation on the isolation of the factors involved in regulation.

Hull Zoölogical Laboratory,
University of Chicago,
May 17, 1904.

LITERATURE CITED.

Agassiz, Alex., '66.—On the Young Stages of a Few Annelids. Ann. Lyc. N. H. N. Y., vol. viii, pp. 303–343.

Brues, C. T., '03.—Reference in Wilson, E. B., '03.

'04.—Biol. Bulletin, vol. vi, No. 6, May, 1904, p. 319.

Caullery et Mesnil, '97.—Études sur la morphologie comparée et la phylogénie des species chez les Spirorbes. Bull. Scient., France, Belg., t. 30, pp. 185–233.

'97.—Sur les Spirorbis; asymétrie de ces Annelides et enchaînment phylogénique du genre. Compt. Rend., t. 124, pp. 48–50.

Claparede and Mecznikow, '69.—Beiträge zur Erkenntnis der Entwicklungsgeschichte der Chætopoden. Zeit. wiss. Zool., Bd. 19, pp. 163–205.

Giard, A., '76.—Note sur l'embryogénie de la Salmacina Dysteri Huxley. Compt. Rend., t. 82, pp. 233–235 et 285–288.

Grube, Ed., '40.—Actinien, Echinodermen und Würme des Adriatischen u. Mittelm. Königsb., p. 64.

Grube, Ed., '62.—Mittheilungen über die Serpulen mit besonderer Berücksichtigung ihrer Deckel. Breslau. Sitzung der Schlesischen Gesellschaft für vaterlandische Kultur am 19 Juni, '61.

'78.—Annulata Semperiana. Mem. de l'Acad. des Sc. de St. Petersbourg. p. 279.

Haswell, W. A., '85.—The Marine Annelids of the Order Serpulea. Some Observations on their Anatomy, with the Characteristics of the Australian Species. Proceedings of the Linnean Soc. of N. S. Wales, vol. ix, part 3.

Leidy, J., '83.—Manayunkia speciosa. Proceed. Acad. Nat. Sc. Philadelphia, 1883, pp. 204–212.

Meyer, Ed., '88.—Studien über den Körperbau der Anneliden. IV. Die Körperform der Serpulaceen und Hermellen. Mitth. aus der Zool. Stat. zu Neapel, viii, Bd.

Milne-Edwards, M., '45.—Observations sur le développement des Annélides. Annales des Sc. Nat. Troisième Serie. Tome troisième.

Morgan, T. H., '02.—Experimental Studies of the Internal Factors of Regeneration in the Earthworm. Archiv. f. Entwicklungsmech. d. Organismen. xiv, Bd. 3 u. 4 Heft.

'04.—Notes on Regeneration. Biol. Bull., vol. vi, No. 4. V. Transpositional or Compensatory Regeneration of the Chelæ in some Crustacea.

U of M

Müller, Fritz, '64.—Für Darwin, pp. 76–77.

Örley, L., '84.—Die Kiemen der Serpulaceen und ihre morphologische Bedeutung. Mitth. aus der Zool. Station zu Neapel, 5 Bd. s. 197.

Pagenstecher, A., '63.—Untersuchungen über niedere Seethiere aus Cette. VII. Entwicklungsgeschichte und Brutpflege von Spirorbis spirillum. Zeit. wiss. Zool., 12 Bd., pp. 486–495.

Przibram, H., '01.—Experimentelle Studien über Regeneration. Archiv. f. Entwicklungsmech. d. Organismen, Bd. 11.

'02.—Experimentelle Studien über Regeneration. Zweite Mittheilung. Crustaceen. Archiv. f. Entwicklungsmech. der Organismen, Bd. 13, Heft 4.

Roule, L., '85.—Notes embryogéniques. Esquisses du développement de la Dasychone lucullana, D. Ch. Revue Sc. N. Montpellier (3), tome 4, p. 463–470.

Salensky, W., '83.—Études sur le développement des Annélides. Première partie. No. 3. Pileolaria. Arch. Biol., tome 4, p. 143.

de St. Joseph, X., '94.—Les Annélides Polychetes des Côtes de Dinard. Serpuliens. Annales des Sc. Nat. Zool., vii serie, t. xvii.

'98.—Les Annélides Polychetes des Côtes de France. Ann. des Sc. Naturelles. Zool., viii serie, t. v, p. 209.

Willemoes-Suhm, R. v., '71.—Biologische Beobachtungen über niedere Meeresthiere. Zeit. wiss. Zool., 21 Bd., pp. 380–396.

Wilson, E. B., '03.—Notes on the Reversal of Asymmetry in the Regeneration of the Chelæ in Alpheus heterochelis. Biol. Bulletin, vol. iv, 1902–03.

Zeleny, C., '02.—A Case of Compensatory Regulation in the Regeneration of Hydroides dianthus. Archiv. für Entwickelungsmechanik der Organismen., xiii, Bd. 4 Heft.

'03.—The Dimensional Relations of the Members of Compound Leaves. Bull. of the N. Y. Botanical Garden, vol. iii, No. 9.

'03.—A Study of the Rate of Regeneration of the Arms in the Brittle-star, Ophioglypha lacertosa. Biol. Bull., vol. vi, No. 1.

From University of Chicago Library in excha

125

UNIV. OF
SEP

THE UNIVERSITY OF CHICAGO
Founded by John D. Rockefeller

Morph. Lab.

Compensatory Regulation

A DISSERTATION SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, DEPARTMENT OF ZOÖLOGY

By
CHARLES ZELENY

May 17, 1904

Reprinted from the Journal of Experimental Zoölogy
Volume II, No. I
Baltimore, April, 1905